Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Silicon-rhodamine isothiocyanate for fluorescent labelling

*Veselin Nasufovic,[1] Patrick Then,[2,3] Fabian Dröge,[2,3] Michael Duong,[1] Christoph Kaether,[4] Benjamin Dietzek,[2,3] Rainer Heintzmann,[2,3] Hans-Dieter Arndt[1]**

[1]Institute of Organic Chemistry and Macromolecular Chemistry, Friedrich Schiller University Jena, Humboldtstr. 10, 07743 Jena, Germany.

[2]Leibniz Institute of Photonic Technology (IPHT), Albert Einstein-Straße 9, 07745 Jena, Germany

[3]Institute of Physical Chemistry and Abbe Center of Photonics, Friedrich Schiller University Jena Helmholtzweg 4, 07743 Jena, Germany

[4]Leibniz Institute on Aging - Fritz Lipmann Institute (FLI), Beutenbergstraße 11, 07745 Jena, Germany

*Author to whom correspondence should be addressed, hd.arndt@uni-jena.de

- Supporting Information -

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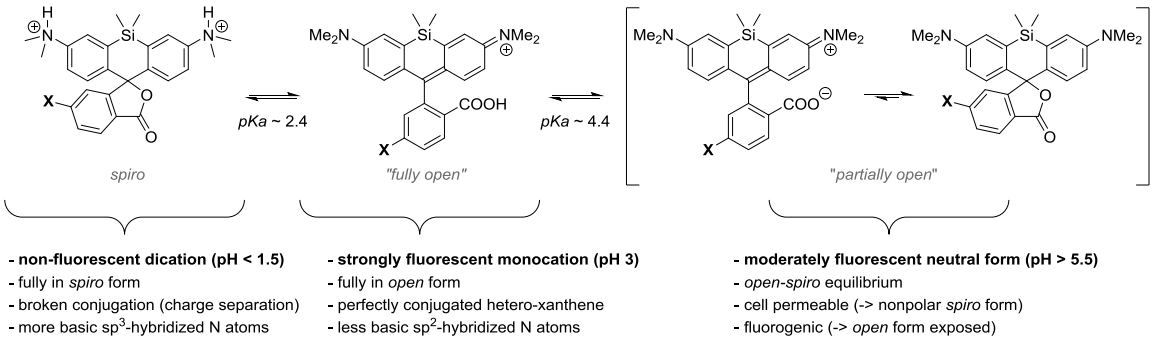
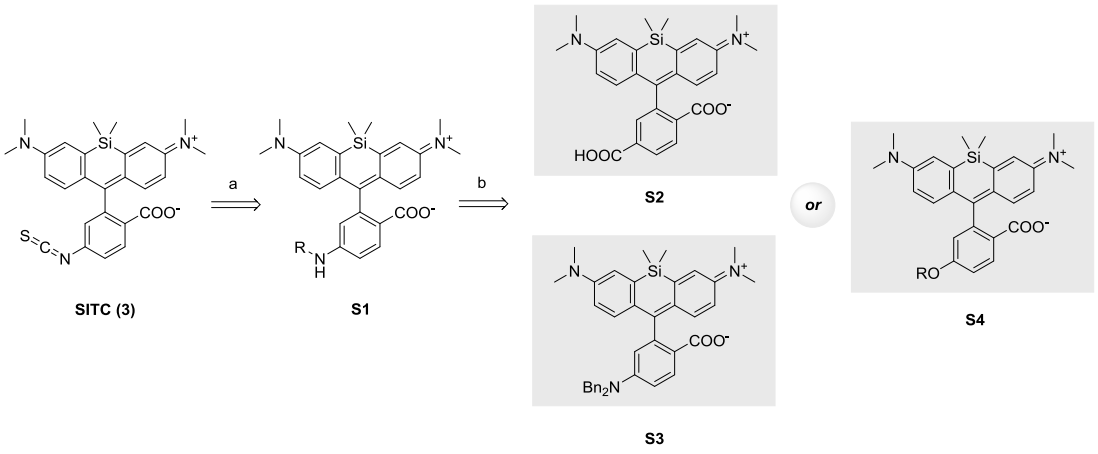
1. Additional Schemes and Figures

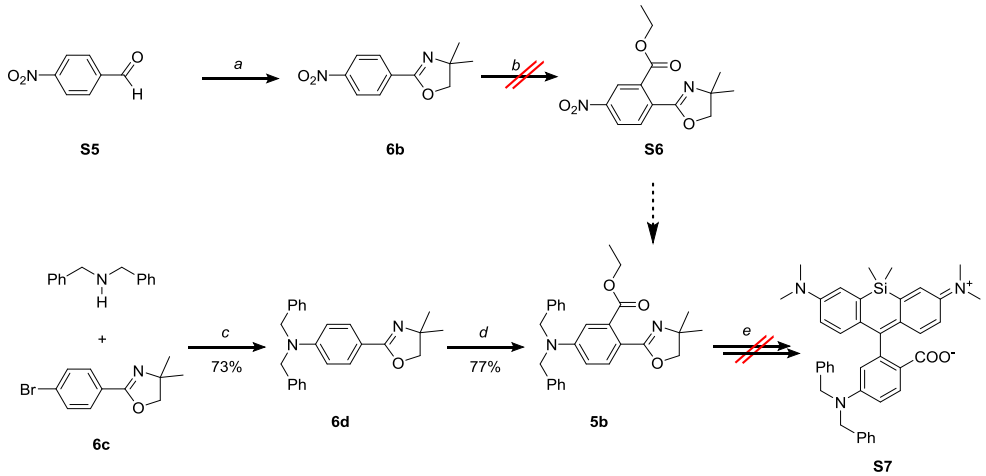
Scheme S1. Protonation states of SiR dyes:



Scheme S2. Retrosynthesis approaches of SITC:

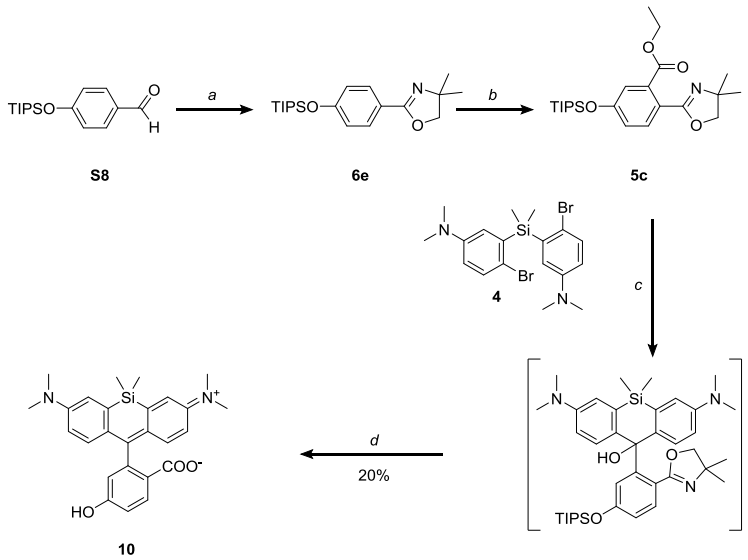


Scheme S3. Toward synthesis of 6-amino-Si-Rhodamine analog:[a]



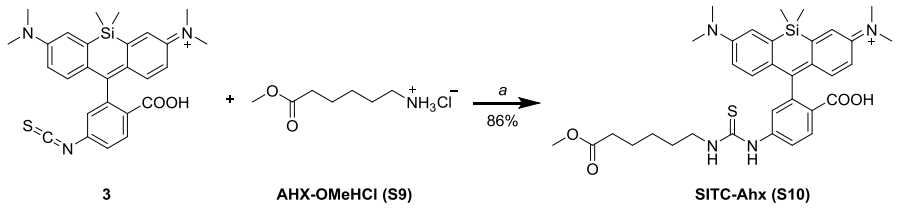
[a]**Reaction conditions:** (a) 2-amino-isobutanol (1.06 equiv.), mol. sieves 4 Å, CH_2Cl_2, 25 °C, 16 h, then: K_2CO_3 (2.7 equiv.), NBS (1.8 equiv.), 8 h, 0 °C to 25 °C, 62%; (b) TMPMgCl·LiCl (2.04 equiv.), 25 °C, 2 h, then: DEPC, -15 °C to 25 °C, 3 h, multiple products; (c) $Pd(dba)_2$ (2 mol%), RuPhos (4 mol%), *t*-BuOK (1.5 equiv.), 16.5 h, 90°C, 73%; (d) TMPMgCl·LiCl (2.04 equiv., 2 h), THF (0 °C), then DEPC (1.96 equiv., added at -40 °C, warmed to 25 °C), 5 h, 77%; (e) **4**, *t*-BuLi, -78 °C, 30 min then **5b**, to 25 °C, 16 h, multiple products formed.

Scheme S4. Synthesis of 6-hydroxy-Si-Rhodamine analog:[a]



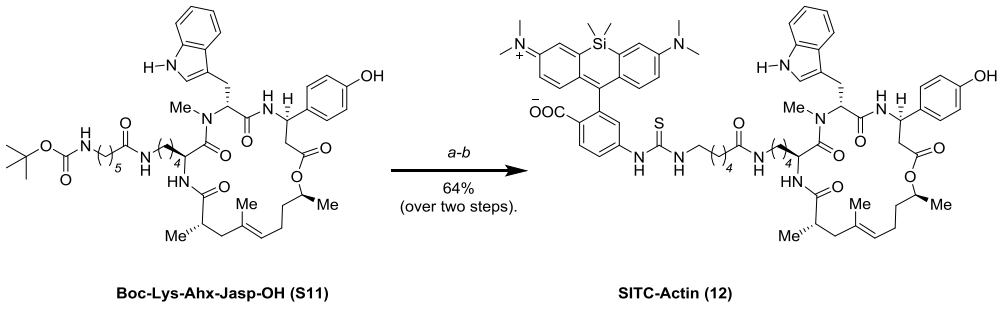
[a]**Reaction conditions: :** (a) 2-amino-isobutanol (1.05 equiv.), mol. sieves 4 Å, CH_2Cl_2, 25 °C, 16 h, then: K_2CO_3 (3 equiv), NBS (1.05 equiv.), 3 h, 0 °C to 25 °C, 62%; (b) TMPMgCl·LiCl (2.04 equiv.), 25 °C, 2 h, then: DEPC (2.1 equiv.), -40 °C to 25 °C, 2.5 h, 73%; (c) **4**, *t*-BuLi (4.2 equiv.), -78 °C, 30 min then **5c** (1 equiv.), to 25 °C, 17 h; (d) 12 M HCl, 23 h, 20% (two steps)

Scheme S5. Synthesis of Ahx conjugate of SITC (SITC-Ahx):[a]



Reagents and conditions: (a) **3** (1 equiv.), Ahx-OMe·HCl (1.2 equiv.), EtN(*i*-Pr)$_2$ (4 equiv.), CH_2Cl_2, 25 °C, 1 h, 86%.

Scheme S6. Synthesis of SITC-Actin dye **12**:



Reagnets and conditions: (a) CH_2Cl_2, HCl in 1,4-dioxane, 0 °C, 2 h; (b) **3** (2 equiv.), EtN(*i*-Pr)$_2$ (4 equiv.), DMF, 0 °C to 25 °C, 16 h, 64% (two steps).

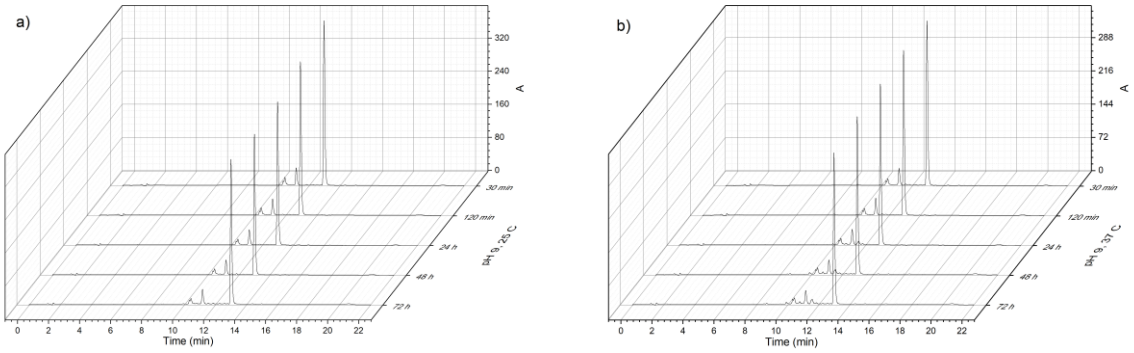


Figure S1. RP-HPLC-monitoring of **SITC stability** in protein labelling buffer: 100 µM of SITC incubated in carbonate buffer (pH 9) / DMSO 49:1 at a) 25 °C and b) 37 °C, after 30 min, 120 min, 24 h, 48 h and 72 h (top-down); SITC at RT 12.98 min.

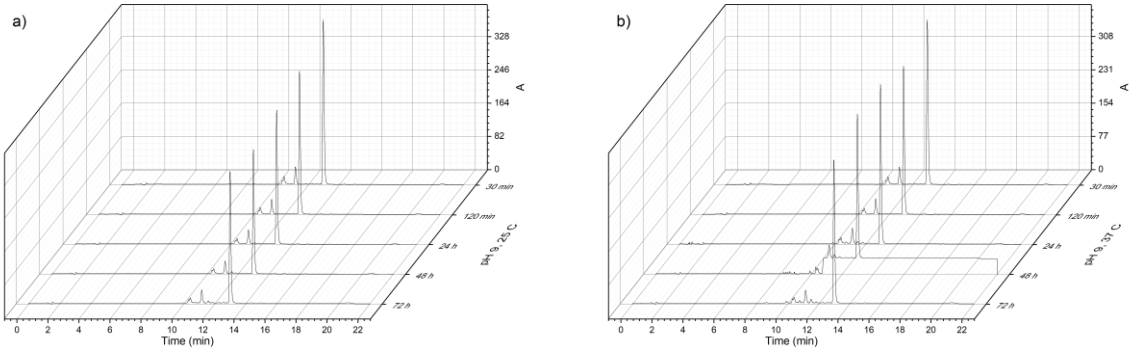


Figure S2. RP-HPLC-monitoring of **SITC reactivity** in protein labelling buffer: 100 µM of SITC incubated in carbonate buffer (pH 9) / DMSO 49:1 with 100 µM of Ahx-OMe·HCl at: a) 25 °C and b) 37 °C, after 30 min, 120 min, 24 h, 48 h and 72 h (top-down); SITC at RT 12.98 min; absence of expected product at RT 11.54 min.

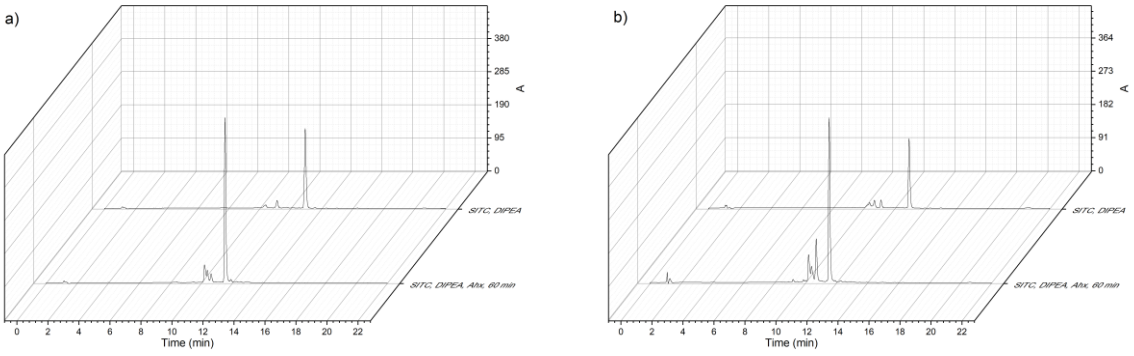


Figure S3. RP-HPLC-monitoring of **SITC reactivity** in organic solvents: 100 µg of SITC dissolved in a) THF and b) DMF (1 mL) with 2 equiv. Ahx-OMe·HCl and 4 equiv. of EtN(*i*-Pr)$_2$; full conversion was observed after 20-30 min, by TLC; SITC at RT 12.98 min; thiocarbamate product at RT 11.54 min.

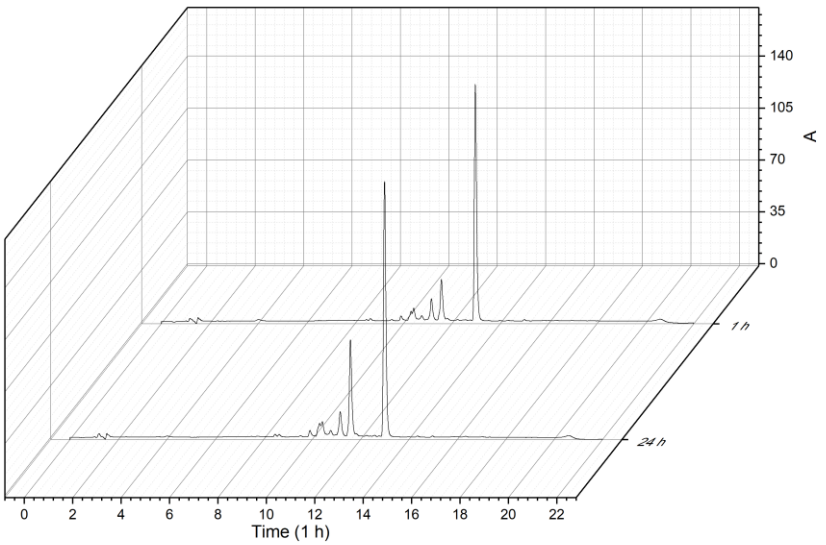


Figure S4. RP-HPLC-monitoring of **SITC reactivity** in protein labelling buffer: 100 µM of SITC incubated in carbonate buffer (pH 9) / DMSO 49:1 with 1 mM of Ahx-OMe·HCl at 37 °C, after 1 h, 24 h (top-down); SITC at RT 12.98 min; thiocarbamate product at RT 11.54 min.

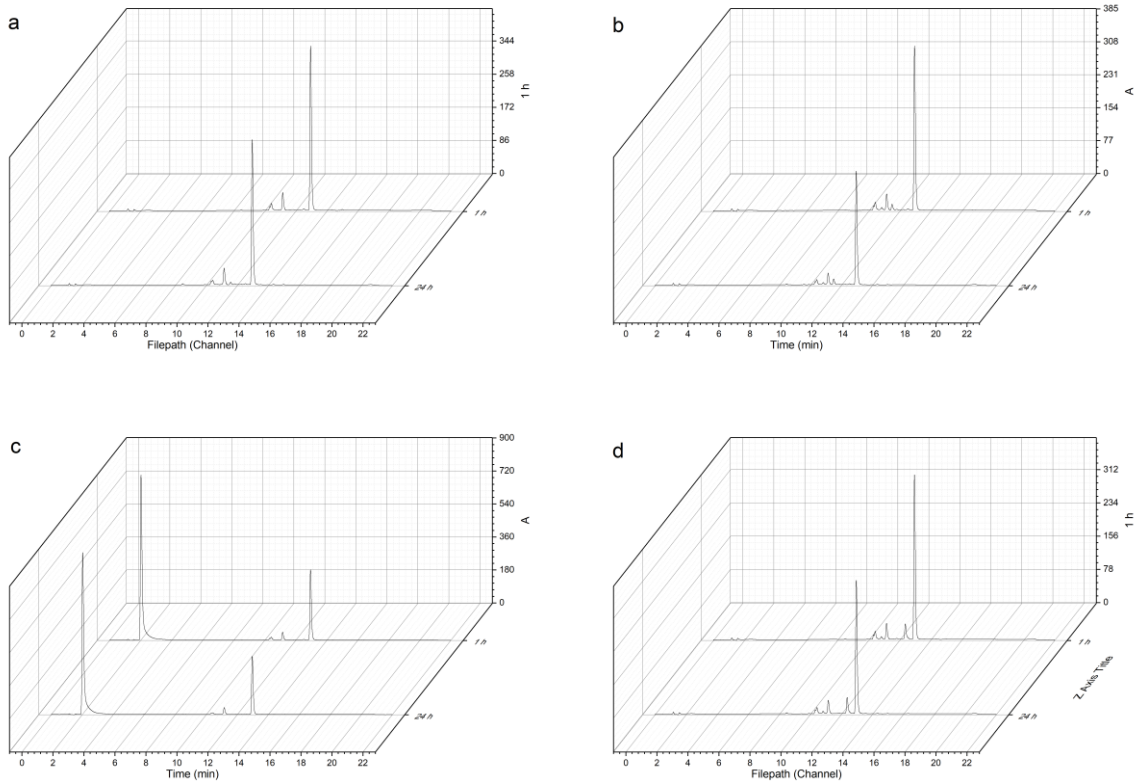


Figure S5. RP-HPLC-monitoring of **SITC reactivity** in **reactivity** in protein labelling buffer: 100 µM of SITC incubated in carbonate buffer (pH 9) / DMSO 49:1 at 37 °C with 200 µM of Ahx-OMe·HCl and: a) 100 µM of TCEP; b) 100 µM of DTT; c) 100 µM of DMAP; d) 100 µM of ethanethiol, after 1 h and 24 h (top-down); SITC at RT 12.98 min; expected thiocarbamate product at RT 11.54 min.

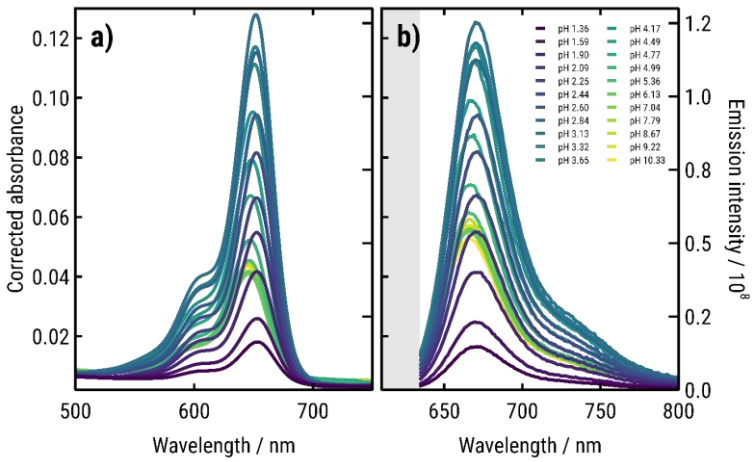


Figure S6. Dilution-corrected pH dependant absorption (a) and emission (b) spectra of SITC-Ahx recorded in aqueous solutions with pH values ranging from 1.36 to pH 10.33. Spectra series were recorded by adding small aliquots of dilute aqueous NaOH into a solution of SITC-Ahx in 0.1 M HCl, both supplemented with 10 vol% DMSO. For details *vide infra*.

Figure S7. Image of purified product from a gram-scale synthesis batch of SiR-COOH (**2**).

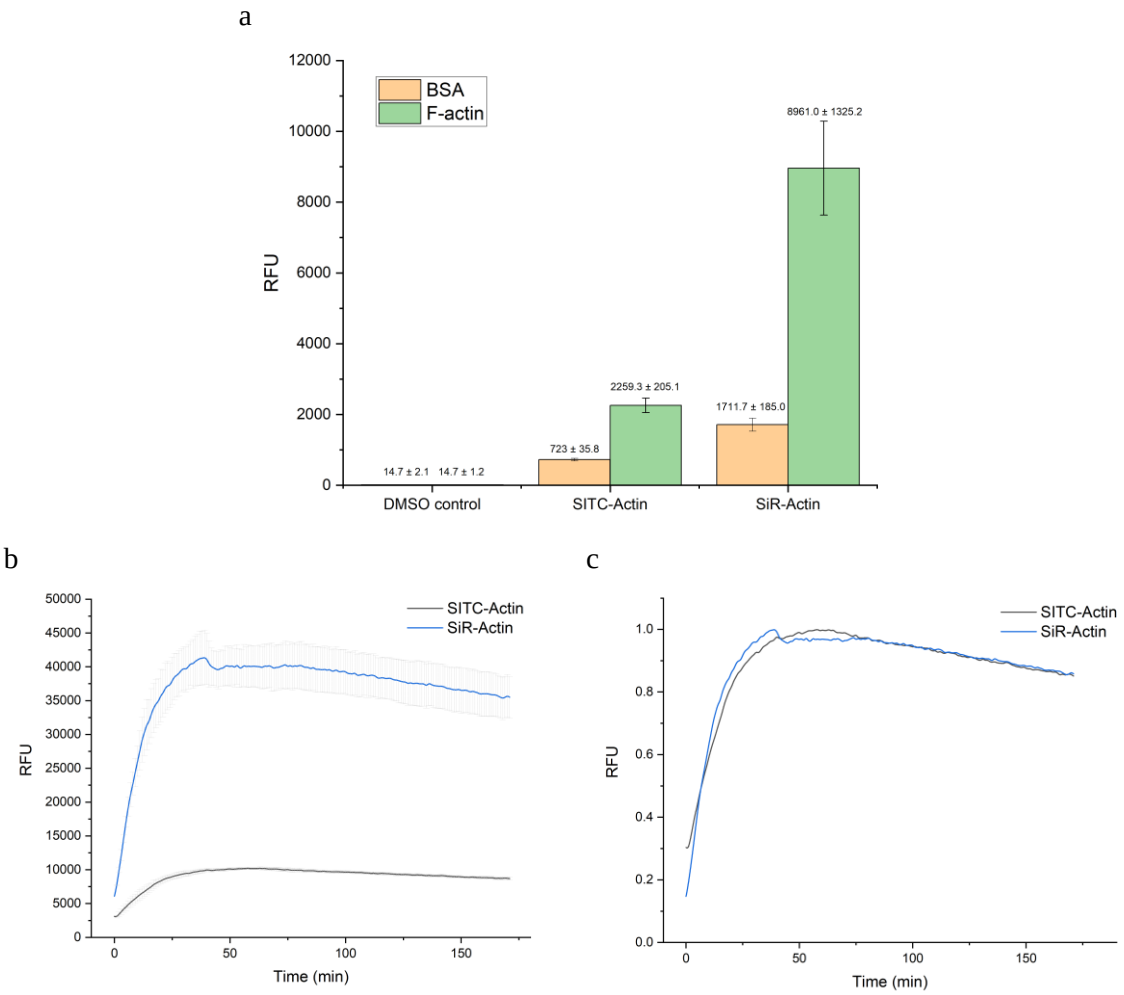


Figure S8. (a) Comparison of emission intensity of SiR-Actin (2.5 µM) and SITC-Actin (2.5 µM) in presence of BSA (5 µM in G-buffer) or in presence of F-Actin (5 µM in G-buffer, high salt conc.) for change in fluorescence intensity ("fluorogenicity"), after incubation for 1 h at 37 °C; (b) increase in emission intensity of SiR-Actin (1.25 µM) and SITC-Actin (1.25 µM) in F-actin (5 µM in G-buffer, high salt conc., excitation at 620 nM, emission 670 nm); (c) normalized to data from graph b. Actin de/polymerization was studied like previously described.[1-2]

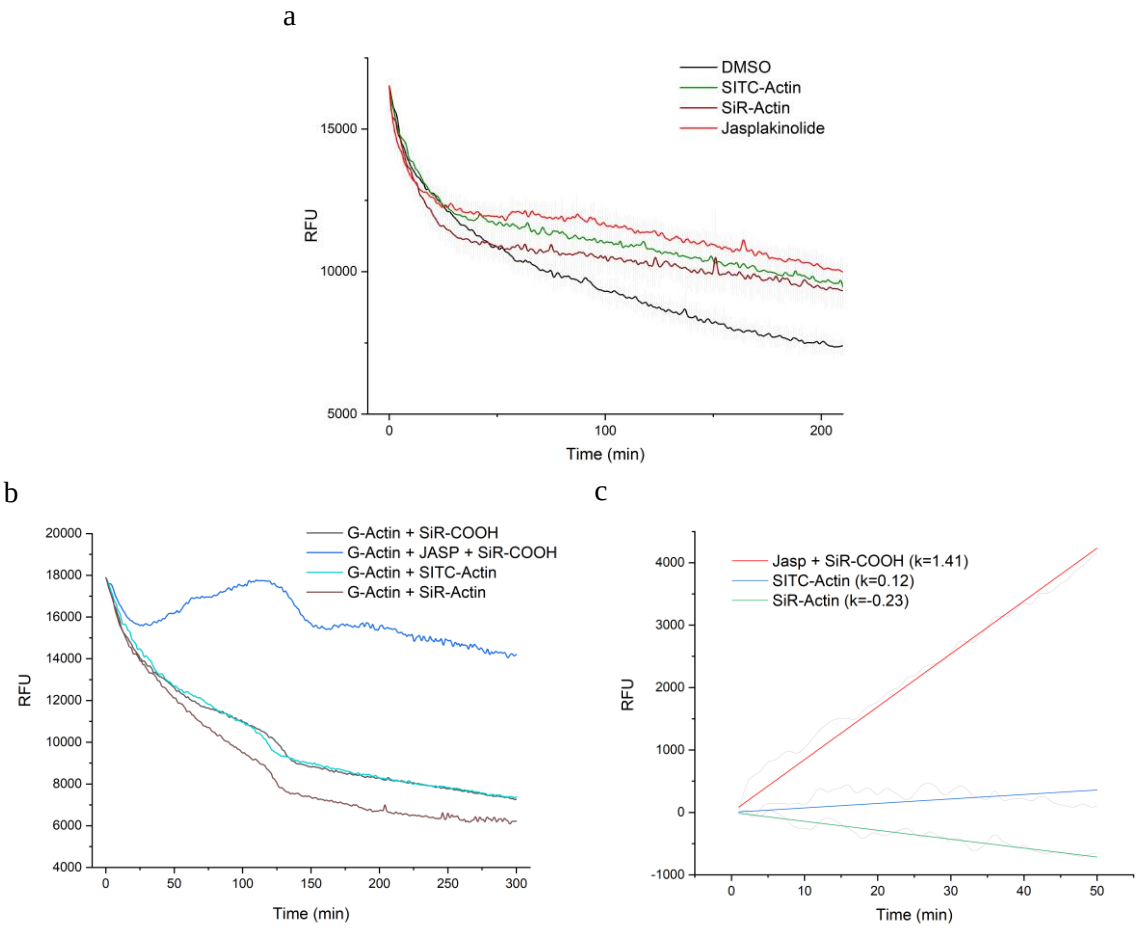
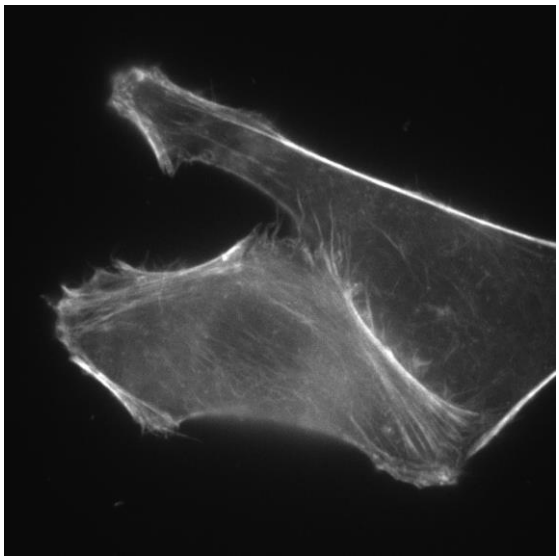
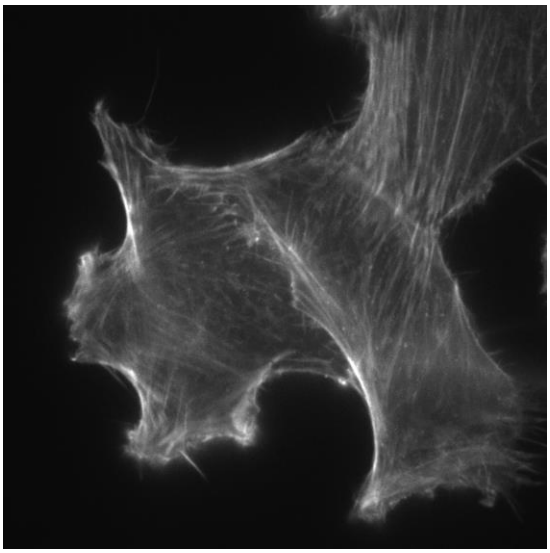


Figure S9. Activity on target (*in vitro*): (a) Comparison of SiR-Actin (20 µM) and SITC-Actin (20 µM) in stabilizing of preformed F-actin (5 µM in G-buffer, high salt conc.) with natural product jasplakinolide (20 µM); (b) potency of SiR-Actin (20 µM) and SITC-Actin (20 µM) for inducing G-actin polymerization (5 µM) in G-buffer at low salt concentration; (c) comparison of potency for induction of G-actin polymerization shown for the initial period, normalized to the fluorescence emission intensity of pyrene-G-actin with DMSO control; linear fitting was applied for comparison of the slopes. Decay (a) or enhancement (b,c) of pyrene-actin fluorescence was monitored (excitation at 360 nm, emission 410 nm). Actin de/polymerization assays were performed like previously described.[1-2]

a



b



c

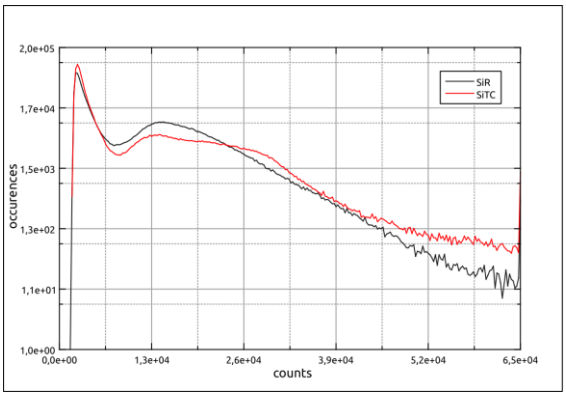


Figure S10. (a) SITC-Actin, (b) Sir-Actin: Hela cells stained overnight (>12 hours) with 150 nM SiTC-Actin and SiR-Actin, respectively. Images have been recorded on a Zeiss Elyra under identical imaging conditions (40% laser power, 200 ms exposure time, em-gain 50); (c) Histogram showing the intensity distribution in the fluorescence images for SiR-Actin (black) and SiTC-Actin (red).

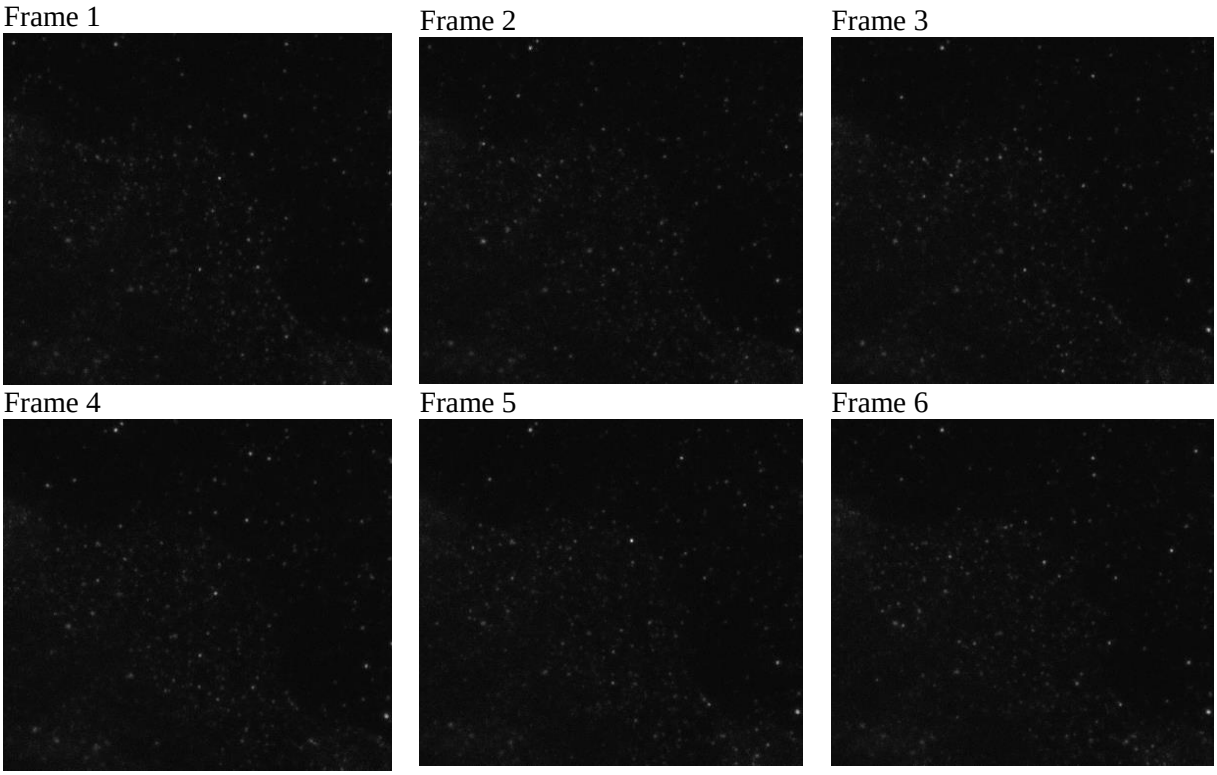


Figure S11. Six blinking frames: fixed HeLa cells labelled with 300 nM SITC-Actin, magnification 160x.

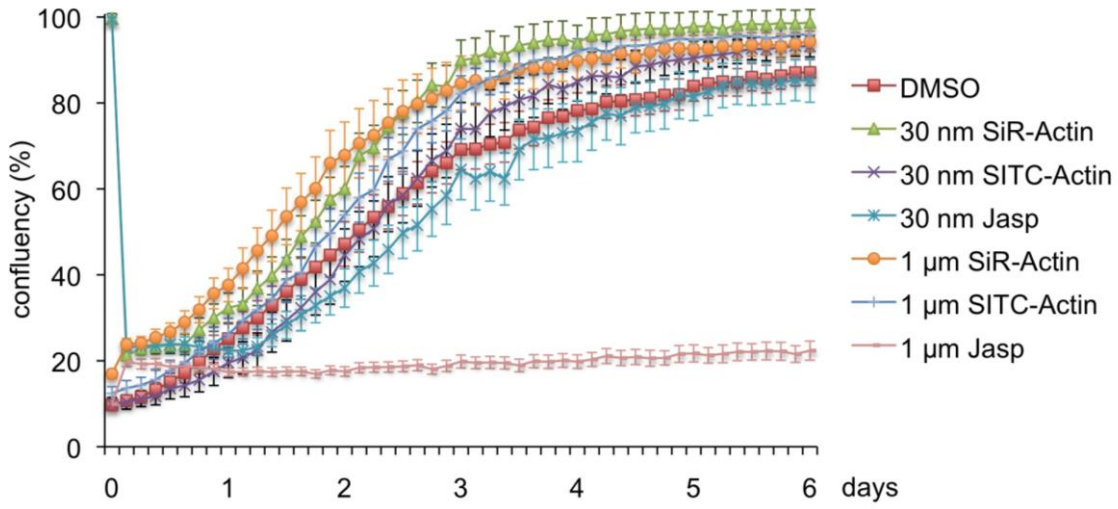


Figure S12. Cytotoxicity of compounds during long-term incubation. Cultured MCF10A cells were plated in 96-well plates, incubated for 24h, treated with compounds as indicated, transferred to an automated incubator-housed imaging system (Incucyte) and cultivated and monitored for 6 days. Every three hours a phase contrast image was generated and the confluency calculated. For each compound and concentration the mean of three technical replicates is displayed. Error bars indicate SD. Shown is a representative experiment from $n = 3$ independent experiments. Note that the peak at 3 h is a technical glitch in one of the conditions. Actin staining similar to HeLa cells indicated MCF-10A cells to be similarly permeable for the tested compounds (data not shown). Growth behaviour was similarly unaffected at 10 µM SIR- or SITC-Actin (data not shown).

2. List of abbreviations

TMP	2,2,6,6-Tetramethylpiperidine
Ahx	6-Aminohexanoic acid
SiR	silico-rhodamine
SITC	silico-rhodamine isothiocyanate
PE	petroleum ether
NBS	*N*-Bromosuccinimide
DEPC	Diethyl pyrocarbonate
TCEP	tris(2-carboxyethyl)phosphine
DMAP	4-Dimethylaminopyridine
DTT	Dithiothreitol
FCC	Flash column chromatography

3. Organic Syntheses

3.1. General information

3.1.1. Reagents

All reagents were obtained from Acros Chemicals, Alfa Aesar, Apollo Scientific, ABCR, Carbolution Chemicals, Carbosynth, Manchester Organics, Merck, Novabiochem, Sigma-Aldrich, TCI Europe, or VWR, and used without further purification. All solvents, if not purchased in purity or dryness suitable, were distilled using standard methods.[3] Tetrahydrofuran (THF) was distilled under a N_2 atmosphere from Na/benzophenone; dichloromethane (CH_2Cl_2) was distilled under a N_2 atmosphere from CaH_2 before use. Other solvents were passed through activated alumina (toluene) or 3Å molecular sieves columns (dimethylformamide - DMF) of a solvent purification system (Pure Solv, Innovative Technology, Inc., USA) by applying N_2 overpressure immediately before use.

All solvents for flash chromatography were distilled prior to use. All solvents used in reactions were anhydrous. Solvents were degassed by employing triple freeze-pump-thaw cycles when necessary, or by purging with N_2 for a minimum 15 minutes. Deionized water was used for all experiments.

3.1.2. Reaction conditions

All reactions were performed in heat dried glassware under N_2 atmosphere if not stated otherwise.

3.1.3. Thin Layer Chromatography (TLC)

TLC was carried out on Merck precoated silica gel plates (60F-254); compounds were visualized using ultraviolet light irradiation at 254 nm and 366 nm or by using the following staining agents (dip, dry & heat development):

Potassium Permanganate: $KMnO_4$ (1 g), K_2CO_3 (6.6 g), 5% NaOH (1.7 mL) in H_2O (90 mL).

Ninhydrin: Ninhydrin (0.3 g) dissolved in *n*-butanol (100 mL) and acetic acid (3 mL).

Ceric Ammonium Molybdate: Ceric ammonium sulfate (0.5 g) and ammonium molybdate (12 g) dissolved in H_2O (235 mL) with conc. H_2SO_4 (15 mL) added.

3.1.4. Silica gel flash liquid chromatography (FCC)

Purifications were performed using silica gel from Macherey & Nagel (particle size 40 – 60 μm) under approximately 0.2-0.6 bar pressure.

3.1.5. NMR spectroscopy

^{1}H- and ^{13}C-NMR spectra were recorded using a Bruker Avance I 250 system (250 MHz for ^{1}H- and 63 MHz for ^{13}C-NMR), a Bruker Fourier 300 system (300 MHz, ^{1}H- and 75 MHz for ^{13}C-NMR), a Bruker Avance I 400 system (400 MHz for ^{1}H- and 101 MHz for ^{13}C-NMR), Bruker Avance 600 system (600 MHz for ^{1}H- and 151 MHz for ^{13}C-NMR) or Avance III HD [500 MHz, probe: BBO (Prodigy)] Spectra were calibrated to appropriate residual solvent peaks (chloroform-*d*, methanol-d_4, MeCN-d_3, DMSO-d_6).[4] Spectra were recorded at 298 K, if not stated otherwise. Chloroform-*d* was stored over molecular sieves in fridge. If mixtures of solvents were used, namely chloroform-*d* and methanol-d_4, spectra were calibrated to chloroform-*d*.

3.1.6. Mass spectrometry

ESI-MS were performed on a Finnigan LCQ spectrometer for monitoring of reaction conditions. Calculated masses were obtained using the software ChemDraw Professional 16. High resolution mass spectrometry (HR-MS) measurements were performed using an LC-coupled MAXIS Impact ESI-TOF spectrometer (Bruker Daltronics, Bremen, Germany).

3.1.7. Analytical reversed-phase high performance liquid chromatography (RP-HPLC)

Analyses were performed on a Varian Prostar system equipped with an autosampler Prostar 410 and a Varian Prostar 335 diode array detector using a EC250/4 Nucleodur C18 Gravity 5 µm (Macherey & Nagel, Düren, Germany) and a Phenomenex Luna 5 µm C8(2) 100 A (250 x 4 mm) column. Linear gradients were employed at 1 mL/min flow rate (A: water, B: acetonitrile).

3.1.8. Preparative reversed-phase HPLC (prep. HPLC)

A Varian Prostar system equipped with a fraction collector prostar 701 and detection at 220 nm (UV/Vis prostar 340) and either a VP 250/21 Nucleodur **C18** Gravity 5 µm column (Macherey & Nagel, Düren, Germany) or a Phenomenex Luna 5µ **C8**(2) 100A, Axia P (250 x 21 mm) column was used. Fractions containing pure product were lyophilized using a Christ Alpha 1-2 LDplus freeze dryer. Linear gradients were employed with A: water, B: acetonitrile.

3.1.9. Fourier transform infrared spectroscopy (FT-IR)

IR spectra were measured by using a Thermo Nicolet Spectrometer FT-IR Avatar 370 fitted with an ATR unit. Spectra were analysed using Spectragryph v1.2.11. The following notations indicate the intensity of the absorption bands: s = strong, m = medium, w = weak.

3.1.10. Melting points

For melting point determination, a Büchi B-545 melting point apparatus and one-side open capillaries were used. All given values are average of three measurements.

3.1.11. Specific optical rotation

Optical rotations were recorded in a Jasco P-2000 polarimeter at 589 nm and at the given temperature (22-24 °C). Path length of cuvettes was *d = 10 mm*. Concentrations are given in g/100 mL solvent if not stated otherwise.

3.2. Specific starting materials

Compounds **4**,[5] 4-(triisopropylsilyloxy)benzaldehyde,[6] Boc-Lys-Ahx-Jasp-OH (**S11**),[2] and **S8**[7] were synthesized according to previously published procedures.

3.2.1. Preparation of reagents

_i_-PrMgCl·LiCl:[8]

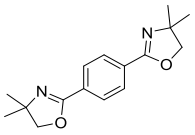
Preactivated Mg turnings (2.82 g, 116 mmol) were placed in a Schlenk flask equipped with reflux condenser. Anhydrous LiCl (4.58 g, 108 mmol) and anhydrous THF (50 mL) were added. Isopropyl chloride (9.70 mL, 106 mmol) was dissolved in anhydrous THF (50 mL) in a separate flask and slowly added dropwise to the stirred suspension of Mg turnings. After approximately 1/10 of isopropyl chloride solution was added, the mixture was heated by using a heat gun until a reaction became apparent. Then, the remainder of the alkyl chloride solution was slowly added at such a rate to entertain a continuous reaction, gradually rising to gentle reflux. After addition was complete, the reaction mixture was stirred at 25 °C for 18 h. 1 mL was withdrawn from the reaction mixture and titrated against I_2 (132 mg, 0.52 mmol) in 1.5 mL of anhydrous THF, indicating a concentration of 0.87 M.

TMPMgCl·LiCl:[8]

To a Schlenk flask charged with *i*-PrMgCl·LiCl solution (103 mL, 89.3 mmol) was added TMP (15.8 mL, 93.7 mmol) dropwise. The resulting mixture was stirred at room temperature for 48 h. For concentration determination, 1 mL was removed and titrated with benzoic acid (106 mg, 0.87 mmol) in 1.5 mL of anhydrous THF by using 4-(phenylazo)-diphenylamine (3 mg) as indicator. A color change from yellow or orange to violet indicated end of titration.[9] Concentrations of 0.7-0.8 M were usually obtained.

3.2.2. Syntheses of oxazolines

1,4-bis(4,4-dimethyl-4,5-dihydrooxazol-2-yl)benzene (6a):



A procedure described for a related compounds was adapted.[10] Terephthalaldehyde (3.035 g, 22.62 mmol, 1 equiv.) and 2-amino-2-methylpropan-1-ol (4.17 g, 46.8 mmol, 2.05 equiv.) were dissolved in anhydrous CH_2Cl_2 (80 mL), to which molecular sieves 4 Å (beads, 8-12 mesh) were previously added (~2 g). The mixture was stirred for 23 h at 25 °C. The flask was cooled to 0 °C and NBS was added in portions (8.08 g, 45.4 mmol, 2.0 equiv.). The mixture was stirred for 10 min at 0°C, the cooling bath was removed, and stirring was continued for 3 h at 25 °C. The reaction mixture was filtered and the solid residue was washed with EtOAc (150 mL) and sat. $NaHCO_3$ (100 mL) solution. The phases were separated, and the aqueous layer was extracted with EtOAc (3 x 50 mL). The organic extracts were combined, dehydrated (Na_2SO_4), and concentrated under reduced pressure. Purification of the residue by FCC (PE/EtOAc 8:2) gave the *bis*-oxazoline **6a** as a colorless solid (5.6 g, 91% yield).

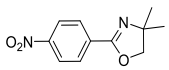
TLC: R_f = 0.45 (PE/EtOAc = 1:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.97 (s, 4H), 4.13 (s, 4H), 1.39 (s, 12H) ppm.

^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): δ = 161.5, 130.5, 128.1, 79.2, 67.8, 28.4 ppm.

Spectral properties corresponded to previously published data.[11]

4,4-dimethyl-2-(4-nitrophenyl)-4,5-dihydrooxazole (6b):



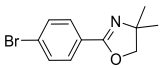
4-nitrobenzaldehyde (2.25 g, 14.9 mmol, 1 equiv.), 2-amino-2-methylpropan-1-ol (1.61 mL, 16.8 mmol, 1.06 equiv.) and molecular sieves 4 Å (~3 g) were dissolved in CH_2Cl_2 (100 mL) and stirred for 16 h at 25 °C. The reaction mixture was then cooled to 0 °C. K_2CO_3 (6.33 g, 45.8 mmol, 2.7 equiv.) and NBS (5.38 g, 30.2 mmol, 1.8 equiv.) were added (NBS added in portions). The mixture was stirred for 10 min at 0 °C and then for 8 h at 25 °C. Solids from reaction mixture were filtered off and the residue washed with EtOAc (~50 mL) and with saturated $NaHCO_3$ solution. The aqueous phase was extracted with EtOAc (3 x ~50 mL). The combined organic extracts were dehydrated (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by FCC (PE/EtOAc 7:3) to give oxazoline **6b** as a colorless solid (2.98 g (91% yield).

TLC: R_f = 0.63 (PE/EtOAc = 1:1).

^{1}H NMR (250 MHz, $CDCl_3$, 297 K) δ = 8.32 – 8.22 (m, 1H), 8.17 – 8.07 (m, 1H), 4.18 (s, 1H), 1.42 (s, 3H) ppm.

Spectral data corresponded to data in previously published literature.[12]

2-(4-Bromophenyl)-4,4-dimethyl-4,5-dihydrooxazole (6c):



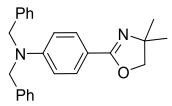
Analogously to the procedure described for compound **6b**, aryl bromide **6c** was synthesized, starting with 4-bromobenzaldehyde (4.30 g, 23.2 mmol, 1 equiv.) and 2-amino-2-methylpropan-1-ol (2.36 mL, 24.7 mmol, 1.06 equiv.) which was stirred for 15 h. After addition of NBS (4.30 g, 24.2 mmol, 1.04 equiv.) at 0 °C, the mixture was let to warm up to 25 °C over 8 h. After work up, product was isolated by FCC using CH_2Cl_2 as eluent to obtain oxazoline 6c as a colorless solid (2.41 g, 41%).

TLC: R_f = 0.73 (PE/EtOAc = 1:1).

^{1}H NMR (250 MHz, $CDCl_3$, 297 K) δ = 7.86 – 7.79 (m, 1H), 7.60 – 7.52 (m, 1H), 4.13 (s, 1H), 1.40 (s, 3H) ppm.

Spectral data corresponded to data in previously published literature.[12]

***N*,*N*-Dibenzyl-4-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)aniline (6d):**



Bromide **6c** (2.00 g, 7.88 mmol, 1 equiv.), RuPhos (0.15 g, 0.32 mmol, 4 mol%) and $Pd(dba)_2$ (0.09 g, 0.16 mmol, 2 mol%) were suspended in toluene (70 mL). Dibenzyl amine (2.28 mL, 11.8 mmol, 1.5 equiv.) and potassium *tert*-butoxide (1.77 g, 15.8 mmol, 2.0 equiv.) were added and the reaction mixture was heated to reflux with stirring for 16.5 h. Volatiles were removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (200 mL), washed with saturated NaCl solution (50 mL), dehydrated with solid Na_2SO_4, and concentrated under reduced pressure. The residue was purified by FCC (PE/EtOAc 7:3), to provide aniline **6d** as a colorless solid (2.11 g, 73% yield).

TLC: R_f = 0.63 (PE/EtOAc = 1:1).

MP: 116 °C

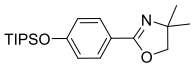
IR (ATR) $\tilde{\nu}$ = 3055 (w), 2970 (w), 2885 (w), 2005 (w), 1955 (w), 1813 (w), 1639 (m), 1608 (m),1519 (m), 1450 (m), 1338 (m), 1292 (m), 1257 (m), 1195 (m), 1064 (m), 964 (m), 736 (s), 686 (s) cm^{-1}.

^{1}H NMR (250 MHz, $CDCl_3$, 297 K) δ = 7.75 (d, J = 9.0 Hz, 2H), 7.39 – 7.17 (m, 11H), 6.72 (d, J = 9.0 Hz, 2H), 4.69 (s, 4H), 4.03 (s, 2H), 1.34 (s, 6H) ppm.

^{13}C{^{1}H} NMR (63 MHz, $CDCl_3$, 297 K) δ = 163.1, 152.1, 138.6, 130.7, 129.7, 128.0, 127.5, 116.9, 112.4, 79.7, 68.1, 54.8, 29.4 ppm.

HRMS (ESI−TOF) m/z $[M+H]^+$ calculated for $C_{25}H_{27}N_2O^+$ 371.2118; found: 371.2128.

4,4-Dimethyl-2-(4-((triisopropylsilyl)oxy)phenyl)-4,5-dihydrooxazole (6e):



Analogously to procedure described for compound **6b**, TIPS-protected phenol **6e** was synthesized starting from 4-(triisopropylsilyloxy) benzaldehyde[6] (2.20 mL, 22.8 mmol, 1 equiv.) and 2-amino-2-methylpropan-1-ol (2.20 mL, 22.8 mmol, 1.05), which was stirred for 16 h. After addition of solid K_2CO_3 (9.01 g, 65.2 mmol, 3.0 equiv.) and NBS (4.06 g, 22.8 mmol, 1.05 equiv.) at 0 °C, the mixture was warmed up to 25 °C and stirred for 2 h. The mixture was filtered and the residue washed with EtOAc (50 mL) and with saturated $NaHCO_3$ solution (30 mL). The aqueous phase was extracted with EtOAc (3x 50 mL). The organic extracts were combined, dehydrated (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by FCC using CH_2Cl_2 as eluent to obtain oxazoline **6e** as a colorless oil (4.67 g, 62%).

TLC: R_f = 0.73 (PE:EtOAc = 1:1).

IR (ATR) $\tilde{\nu}$ = 2947 (m), 2866 (m), 1647 (m), 1604 (m), 1508 (s), 1462 (m), 1350 (m), 1269 (s), 1165 (m), 1060 (m), 995 (m), 906 (s), 844 (m), 732 (s), 682 (s) cm^{-1}.

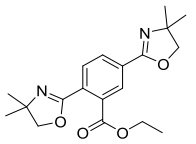
^{1}H NMR (400 MHz, $CDCl_3$, 297 K) δ = 7.85 – 7.80 (m, 2H), 6.91 – 6.87 (m, 2H), 4.08 (s, 2H), 1.38 (s, 6H), 1.32 – 1.22 (m, 3H), 1.11 (d, J = 7.3 Hz, 18H) ppm.

^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$, 297 K) δ = 162.0, 158.9, 129.9, 120.9, 119.7, 79.0, 67.4, 28.5, 17.9, 12.7 ppm.

HRMS (ESI−TOF) m/z $[M+H]^+$ calculated for $C_{20}H_{34}NO_2Si^+$ 348.2353; found: 348.2361.

3.2.3. Directed ortho metalation enabled synthesis of esters

Ethyl 2,5-bis(4',4'-dimethyl-4',5'-dihydrooxazol-2'-yl)benzoate (5a):



Bis-oxazoline **6a** (4.06 g, 14.9 mmol, 1 equiv.) was dissolved in anhydrous THF (100 mL) and TMPMgCl·LiCl (28.2 mL, 1.06 M, 29.8 mmol, 2 equiv.) was added at ambient temperature. After stirring for 2 h at ambient temperature, the mixture was cooled to -15 °C and DEPC (6.50 mL, 44.7 mmol, 3 equiv.) was added dropwise. After stirring for 20 min at -15°C, the mixture was warmed up slowly and stirred for additional 16 h at ambient temperature. Saturated NH_4Cl solution (50 mL) was added with mixing, the layers were separated, and the aqueous layer was extracted with EtOAc (3 x 100 mL). Organic extracts were combined, dehydrated (Na_2SO_4), and concentrated under reduced pressure. The residue was purified using FCC (PE:EtOAc = 8:2) to give ester **5a** as light yellow oil that solidified upon standing at 0°C (3.98 g, 77% yield).

TLC: R_f = 0.38 (PE:EtOAc = 1:1).

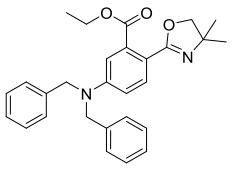
IR (ATR) $\tilde{\nu}$ = 2945 (m), 2913 (m), 2189 (w), 2150 (w), 2019 (w), 1688 (m), 1587 (w), 1548 (w), 1429 (w), 1337 (s), 1285 (s), 1225 (s), 1153 (m), 1118 (m), 1033 (s), 999 (s), 942 (s), 883 (m), 802 (w), 767 (w), 635 (w), 600 (w) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$, 297 K) δ = 8.26 (d, *J* = 1.5 Hz, 1H), 8.06 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.80 (d, *J* = 8.0 Hz, 1H), 4.40 – 4.31 (m, 2H), 4.12 (d, *J* = 11.3 Hz, 4H), 1.38 (dd, *J* = 12.2, 4.7 Hz, 16H) ppm.

^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$, 297 K) δ = 167.1, 161.6, 160.7, 132.8, 130.5, 130.2, 130.1, 129.9, 128.6, 79.8, 79.3, 68.2, 68.0, 61.6, 28.4, 28.1, 14.2 ppm.

HRMS (ESI−TOF) m/z $[M + H]^+$ calculated for $C_{19}H_{25}N_2O_4^+$ 345.1809; found: 345.1811.

Ethyl 5-(dibenzylamino)-2-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)benzoate (5b):



Ester **5b** was synthesized as described for **5a**, but starting from oxazoline **6d** (0.49 g, 1.32 mmol, 1 equiv.) with TMPMgCl·LiCl (3.10 mL, 2.64 mmol, 2.04 equiv.) as a base, stirred for 2 h and DEPC (0.38 mL, 2.64 mmol, 1.96 equiv.). After addition of DEPC, the mixture was stirred for 5 h. FCC (PE/EtOAc 9:1) gave ester **5b** as a colorless solid (0.45 g, 77% yield).

TLC: R_f = 0.55 (PE:EtOAc = 1:1).

MP = 122 °C.

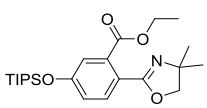
IR (ATR) $\tilde{\nu}$ = 2962 (w), 2924 (w), 1948 (w), 1813 (w), 1724 (s), 1643 (m), 1604 (s), 1554 (m), 1516 (m), 1427 (m), 1357 (s), 1237 (m), 1029 (m), 960 (s), 813 (m), 729 (s), 690 (s) cm^{-1}.

^{1}H NMR (250 MHz, $CDCl_3$, 297 K) δ = 7.64 (d, *J* = 8.7 Hz, 1H), 7.40 – 7.17 (m, 10H), 6.97 (d, *J* = 2.7 Hz, 1H), 6.77 (dd, *J* = 8.8, 2.7 Hz, 1H), 4.71 (s, 4H), 4.30 (q, *J* = 7.1 Hz, 2H), 4.03 (s, 2H), 1.47 – 1.21 (m, 9H) ppm.

^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$, 297 K) δ = δ 169.1, 162.0, 150.5, 137.3, 134.6, 131.3, 128.8, 127.2, 126.6, 114.9, 113.3, 111.5, 79.3, 67.5, 61.4, 53.8, 28.3, 14.1 ppm.

HRMS (ESI−TOF) m/z $[M + H]^+$ calculated for $C_{28}H_{31}N_2O_3^+$ 443.2329; found: 443.2336.

Ethyl 2-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)-5-((triisopropylsilyl)oxy)benzoate (5c):



Oxazoline **6e** (0.39 g, 1.12 mmol, 1 equiv.) was dissolved in anhydrous THF (20 mL) and TMPMgCl·LiCl (2.65 mL, 2.31 mmol, 2.06 equiv.) was added dropwise at 25 °C. After stirring for 2 h at room temperature, the mixture was cooled to -40 °C and DEPC (0.34 mL, 2.35 mmol, 2.1 equiv.)

was added dropwise. The mixture was stirred at -40 °C for 20 min and then 2.5 h at 25 °C. Saturated NH_4Cl solution was added (20 mL) with mixing, layers were separated, and the aqueous layer was extracted with EtOAc (3 x 30 mL). The organic extracts were combined, dehydrated (Na_2SO_4), and concentrated under reduced pressure. FCC (PE/EtOAc 8:2) provided ester **5c** as a colorless oil (0.34 g, 73% yield).

TLC: R_f = 0.40 (PE:EtOAc = 8:2).

IR (ATR) $\tilde{\nu}$ = 2947 (w), 2870 (w), 1732 (m), 1654 (m), 1604 (m), 1500 (m), 1462 (m), 1350 (m), 1303 (s), 1246 (m), 1226 (m), 1095 (m), 1045 (m), 964 (s), 879 (m), 844 (m), 783 (w), 744 (m), 686 (s) cm^{-1}.

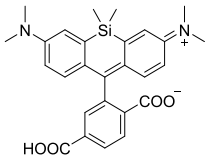
^{1}H NMR (400 MHz, $CDCl_3$, 297 K) δ = 7.65 (d, J = 8.4 Hz, 1H), 7.17 (d, J = 2.5 Hz, 1H), 6.97 (dd, J = 8.5, 2.5 Hz, 1H), 4.35 (q, J = 7.1 Hz, 2H), 4.08 (s, 2H), 1.42 – 1.33 (m, 9H), 1.33 – 1.22 (m, 3H), 1.11 (d, J = 7.3 Hz, 18H) ppm.

^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$, 297 K) δ = 167.7, 162.1, 157.9, 134.2, 131.5, 121.8, 120.6, 120.2, 79.6, 67.8, 61.5, 28.2, 17.9, 14.2, 12.6 ppm.

HRMS (ESI−TOF) m/z $[M + H]^+$ calculated for $C_{23}H_{38}NO_4^+$ 420.2565; found: 420.2575.

3.2.4. Synthesis of Si-rhodamines

4-Carboxy-2-(7-(dimethylamino)-3-(dimethyliminio)-5,5-dimethyl-3,5-dihydrodibenzo[b,e]silin-10-yl)benzoate (2):



A previously reported procedure[13] was adapted. Bis-Br-Arylsilane **4** (0.87 g, 1.91 mmol) was dissolved in anhydrous THF (50 mL) and cooled to -78 °C. *t*-BuLi (4.72 mL, 1.7 M, 8.02 mmol, 4.2 equiv.) was added dropwise and the mixture was stirred at -78 °C with TLC monitoring, until Li-Br exchange was complete (30 min). Ester **5a** (0.66 g, 1 equiv. 1.91 mmol) dissolved in anhydrous THF (30 mL) was then added dropwise over 20 min, keeping the temperature at -78 °C. After additional stirring at -78 °C for 40 min, the cooling bath was removed and the mixture was stirred for 16 h at ambient temperature. Acetic acid (1 mL) was added and volatiles were removed under reduced pressure. The residue was dissolved in 6 M HCl (50 mL) and heated to 90 °C with stirring for 24 h. The mixture was cooled to ambient temperature, the pH was adjusted to 2 by adding 2 M NaOH dropwise (pH meter), and extracted with CH_2Cl_2 (10 x 100 mL). The organic extracts were combined, dehydrated (Na_2SO_4), and concentrated under reduced pressure. FCC (CH_2Cl_2/MeOH 100:0 → 9:1), gave diacid **2** as a blue resinous solid (614 mg, 68% yield). When the procedure was repeated with 1.61 g of ester **5a** (4.65 mmol), 1.10 g of product was obtained (50% yield).

TLC: R_f = 0.55 (CH_2Cl_2:MeOH = 9:1).

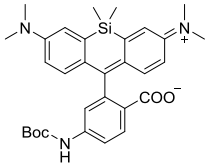
^{1}H NMR (400 MHz, MeOH-d_4, 297 K) δ = 8.17 (d, J = 7.9 Hz, 1H), 7.96 (d, J = 7.9 Hz, 1H), 7.81 (s, 1H), 6.98 (t, J = 2.1 Hz, 2H), 6.68 (d, J = 8.9 Hz, 2H), 6.62 – 6.43 (m, 2H), 2.86 (d, J = 4.2 Hz, 12H), 0.60 (s, 3H), 0.50 (s, 3H) ppm.

^{13}C{^{1}H} NMR (101 MHz, MeOH-d_4, 297 K) δ = 171.80, 155.95, 151.22, 138.13, 132.15, 131.24, 130.75, 129.36, 126.82, 126.78, 118.05, 114.87, 40.48, 40.46, 0.32, -1.14 ppm.

HRMS (ESI−TOF) m/z $[M]^+$ calculated for $C_{27}H_{29}N_2O_4Si^+$ 473.1891; found: 473.1898.

Spectral data corresponded to previously published data.[13-14]

4-((*tert*-Butoxycarbonyl)amino)-2-(7-(dimethylamino)-3-(dimethyliminio)-5,5-dimethyl-3,5-dihydrodibenzo[b,e]silin-10-yl)benzoate (8):



Diacid **2** (800 mg, 1 equiv., 1.69 mmol) was dissolved in 100 mL of toluene/*t*-butanol mixture (1:1). EtN(*i*-Pr)$_2$ (872 mg, 1.1 mL, 4 equiv. 6.76 mmol) was added followed by addition of DPPA (465 mg, 363 µl, 1 equiv., 1.69 mmol). The mixture was heated to 90 °C for 16 h with stirring, turning from blue to yellow and finally to red color. After cooling, volatiles were removed under reduced pressure. The residue was dissolved in water (100 mL), the pH was adjusted to 2-3 using 1 M aqueous solution of HCl, and the mixture was extracted with CH_2Cl_2 (5 x 100 mL). The organic extracts were combined, dehydrated (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by FCC (CH_2Cl_2:MeOH = 98:2) to give protected aniline **8** as yellow to green solid (579 mg, 63% yield)

TLC: R_f = 0.43 (CH_2Cl_2:MeOH = 95:5).

MP: 188 °C (decomp.).

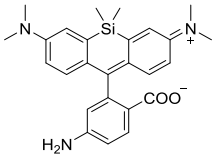
IR (ATR) $\tilde{\nu}$ = 2394 (w), 2193 (w), 2134 (w), 2012 (w), 1975 (w), 1639 (m), 1568 (m), 1465 (s), 1413 (s), 1301 (s), 1201 (s), 1081 (s), 997 (m), 908 (m), 800 (s), 715 (s), 626 (m) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$/MeOH-d_4, 297 K) δ = 8.23 (s, 1H), 8.06 – 7.72 (m, 3H), 7.42 (t, J = 3.5 Hz, 2H), 7.31 – 7.19 (m, 2H), 7.04 (dt, J = 9.7, 3.6 Hz, 2H), 3.44 – 3.33 (m, 12H), 1.90 (dd, J = 4.7, 2.3 Hz, 9H), 1.15 – 1.05 (m, 3H), 1.06 – 0.94 (m, 3H).

^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$/MeOH-d_4, 297 K) δ = 171.6, 156.4, 152.7, 149.3, 149.2, 144.9, 136.6, 131.5, 131.5, 128.1, 128.0, 125.8, 119.8, 118.9, 116.5, 116.4, 113.5, 113.4, 112.7, 92.2, 80.6, 39.9, 39.9, 27.8, 27.8, -0.1, -0.1, -2.0 ppm.

HRMS (ESI−TOF) m/z $[M]^+$ calculated for $C_{31}H_{38}N_3O_4Si^+$ 544.2626; found: 544.2629.

4-amino-2-(7-(dimethylamino)-3-(dimethyliminio)-5,5-dimethyl-3,5-dihydrodibenzo[b,e]silin-10-yl)benzoate (9):



Carbamate **8** (500 mg, 0.92 mmol) was dissolved in CH_2Cl_2 (70 mL) and cooled to 0 °C. 30 mL of anhydrous TFA was added and reaction mixture stirred at 0 °C for 3 h. Volatiles were evaporated under reduced pressure, the residue was dissolved in water (30 mL) and the pH was adjusted to 4 by using 2 M NaOH solution, and extracted with CH_2Cl_2 (3 x 100 mL). The organic extracts were combined, dehydrated (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by FCC (CH_2Cl_2/MeOH 98:2) to give aniline 9 as a green solid (322 mg, 79% yield).

TLC: R_f = 0.39 (CH_2Cl_2:MeOH = 95:5).

MP: 292 °C.

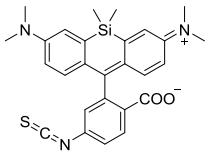
IR (ATR) $\tilde{\nu}$ = 3415 (w), 3269 (w), 2830 (w), 2393 (w), 2193 (w), 2149 (w), 2013 (w), 1962 (w), 1815 (w), 1663 (w), 1525 (m), 1426 (m), 1385 (m), 1298 (s), 1260 (s), 1196 (s), 1141 (s), 993 (m), 935 (m), 877 (s), 798 (s), 746 (s), 666 (s), 617 (s) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$, 297 K) δ = (400 MHz,) δ 7.70 (d, J = 8.2 Hz, 1H), 6.99 – 6.87 (m, 4H), 6.68 (dd, J = 8.3, 2.0 Hz, 1H), 6.60 (dd, J = 9.0, 2.9 Hz, 2H), 6.31 (d, J = 1.9 Hz, 1H), 2.96 (s, 12H), 0.62 (s, 3H), 0.58 (s, 3H) ppm.

^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$, 297 K) δ = 171.1, 158.2, 152.0, 149.3, 136.2, 132.5, 128.6, 127.2, 116.4, 116.3, 115.7, 113.8, 108.2, 90.3, 40.4, 0.4, -0.9 ppm.

HRMS (ESI−TOF) m/z $[M]^+$ calculated for $C_{26}H_{30}N_3O_2Si^+$ 444.2102; found: 444.2106.

2-(7-(Dimethylamino)-3-(dimethyliminio)-5,5-dimethyl-3,5-dihydrodibenzo[b,e]silin-10-yl)-4-isothiocyanatobenzoate (3):



Aniline **9** (300 mg, 0.68 mmol, 1 equiv.) was dissolved in CH_2Cl_2 (30 mL) at 25 °C and Et_3N (206 mg, 278 µL, 2.04 mmol, 3 equiv.) was added. After stirring for 20 min thiophosgene (116 mg, 77 µL, 1.05 mmol, 1.5 equiv.) was added and stirring was continued with TLC monitoring. Full conversion was reached after 3 h. The mixture was diluted with CH_2Cl_2 (70 mL), quickly washed with sat. aqueous $NaHCO_3$ solution (2 x 20 mL), dehydrated (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by FCC (CH_2Cl_2/isopropanol 98:2) to give isothiocyanate **3** as a brown to light green solid (238 mg, 72%).

TLC: R_f = 0.55 (CH_2Cl_2:MeOH = 95:5).

MP: 134 °C (decomp.).

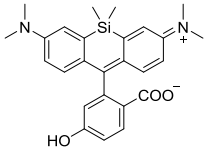
IR (ATR) $\tilde{\nu}$ = 2400 (w), 2191 (w), 2017 (s), 1633 (w), 1566 (m), 1534 (m), 1409 (m), 1295 (m), 1190 (s), 1139 (s), 998 (m), 903 (m), 799 (s), 713 (s), 626 (m) cm^{-1}.

^{1}H NMR ^{1}H NMR (400 MHz, $CDCl_3$, 297 K) δ = 8.00 – 7.89 (m, 1H), 7.32 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.05 (d, *J* = 1.6 Hz, 1H), 6.97 (d, *J* = 2.9 Hz, 2H), 6.83 (d, *J* = 8.9 Hz, 2H), 6.62 (dd, *J* = 9.0, 2.9 Hz, 2H), 3.00 (s, 12H), 0.67 (s, 2H), 0.61 (s, 3H) ppm.

^{13}C{^{1}H} NMR ^{13}C NMR (101 MHz, $CDCl_3$, 297 K) δ = 170.0, 157.5, 149.8, 139.6, 137.3, 136.8, 131.3, 128.5, 127.5, 126.7, 125.1, 121.8, 116.9, 114.0, 91.6, 40.8, 40.6, 0.8, 0.6 ppm.

HRMS (ESI−TOF) m/z $[M]^+$ calculated for $C_{27}H_{28}N_3O_2SSi^+$ 486.1666; found: 486.1674.

2-(7-(Dimethylamino)-3-(dimethyliminio)-5,5-dimethyl-3,5-dihydrodibenzo[b,e]silin-10-yl)-4-hydroxybenzoate (10):



Silane **4** (0.87 g, 1.91 mmol, 1 equiv.) was dissolved in anhydrous THF (50 mL) and cooled to –78 °C. *t*-BuLi (4.72 mL, 1.7 M, 8.02 mmol, 4.2 equiv.) was added dropwise. The reaction mixture was stirred at -78 °C for 1 h. Ester **5c** (0.80 g, 1.91 mmol) was dissolved in anhydrous THF in a separate flask and the solution was added dropwise to the reaction mixture *via cannula* over 10 min. The mixture was stirred at –78 °C for 40 min and then warmed to 25 °C and stirred for 17 h. Acetic acid (1 mL) was added, volatiles were evaporated to dryness, the residue was dissolved in 12 M aqueous HCl (50 mL), and heated to reflux with stirring for 23 h. The mixture was cooled to ambient temperature, the pH was adjusted to 2 with 2 M NaOH (controlled by using a pH meter), and extracted with CH_2Cl_2 (10 x 50 mL). The combined organic extracts were dehydrated (Na_2SO_4) and concentrated under reduced pressure. The residue was purified using preparative HPLC (C18) to give 206 mg (20%) of phenol **10** as a blue solid.

Method used for preparative HPLC: Isocratic 2 min (30% MeCN in H_2O) + 1%AcOH, → gradient over 38 min (30-100% MeCN in H_2O) + 1% AcOH, → 10 min 100% MeCN + 1% AcOH. Flow 25 mL/min.

TLC: R_f = 0.65 (CH_2Cl_2:MeOH = 9:1).

MP: 312 °C

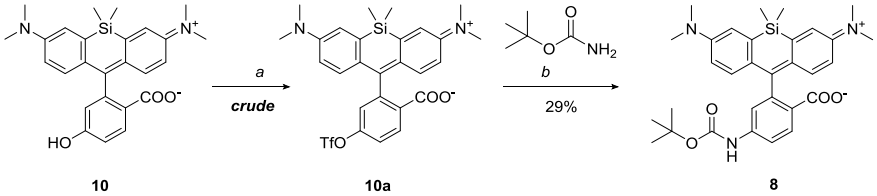
IR (ATR) $\tilde{\nu}$= 3205 (b), 2893 (w), 2804 (w), 2160 (w), 2021 (w), 1701 (m), 1593 (m), 1473 (m), 1357 (m), 1276 (s), 1234 (m), 1180 (m), 1130 (m), 1060 (m), 875 (m), 840 (s), 790 (m), 756 (s), 729 (m), 694 (s) cm^{-1}.

^{1}H NMR (250 MHz, Chloroform-*d*, 297 K) δ = 7.71 (d, *J* = 8.2 Hz, 1H), 6.93 (dd, *J* = 27.3, 5.8 Hz, 5H), 6.70 (s, 1H), 6.57 (dd, *J* = 8.9, 2.7 Hz, 2H), 2.97 (s, 12H), 0.61 (d, *J* = 9.5 Hz, 6H) ppm.

$^{13}C\{^1H\}$ NMR (63 MHz, Chloroform-*d*, 297 K) δ = 172.4, 163.0, 158.1, 150.2, 137.8, 132.8, 129.2, 128.3, 119.4, 118.4, 117.6, 114.4, 111.8, 41.3, 1.3, -0.6 ppm.

HRMS (ESI−TOF) m/z $[M + H]^+$ calculated for $C_{26}H_{29}N_2O_3Si^+$ 445.1942; found: 445.1951.

4-((*tert*-Butoxycarbonyl)amino)-2-(7-(dimethylamino)-3-(dimethyliminio)-5,5-dimethyl-3,5-dihydrodibenzo[b,e]silin-10-yl)benzoate (8):

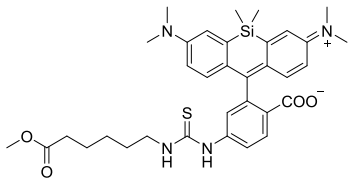


Phenol **10** (69 mg, 0.16 mmol) was dissolved in anhydrous CH_2Cl_2 (8 mL) and Et_3N (0.07 mL, 0.47 mmol) was added dropwise with stirring at 0 °C. The mixture was stirred for 5 min, Tf_2O (0.06 mL, 0.31 mmol) was added, and the mixture was stirred for 1 h at 0 °C. CH_2Cl_2 (50 mL) was added, the mixture was washed with water (2 x 10 mL), dehydrated (Na_2SO_4), and concentrated under reduced pressure to obtain the crude triflate (**10a**) that was used without further purification.

The crude triflate was dissolved in anhydrous 1,4-dioxane (15 mL) and Pd (dppf)Cl_2·CH_2Cl_2 (28 mg, 0.03 mmol, 20 mol%), Cs_2CO_3 (193 mg, 0.59 mmol, 3 equiv.), *tert*-butyl carbamate (66 mg, 0.57 mmol, 3 equiv.) and Xantphos (45 mg, 0.08 mmol, 50 mol%) were added. The mixture was heated to reflux with stirring for 18 h. The mixture was cooled to ambient temperature, concentrated under reduces pressure, and redissolved in CH_2Cl_2 (30 mL). The mixture was washed with saturated NaCl solution (10 mL), dehydrated (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by FCC (CH_2Cl_2/MeOH 199: 1) to provide carbamate **8** as light-yellow solid (25 mg, 29% yield), that was identical to the material obtained from the alternative procedure by spectroscopic data.

3.2.5. Synthesis of conjugates

2-(7-(Dimethylamino)-3-(dimethyliminio)-5,5-dimethyl-3,5-dihydrodibenzo[b,e]silin-10-yl)-4-(3-(6-methoxy-6-oxohexyl)thioureido)benzoate (SITC-Ahx):



6-Aminohexanoic acid methyl ester hydrochloride (7 mg, 0.036 mmol, 1.2 equiv.) was dissolved in CH_2Cl_2 (5 mL) and EtN(*i*-Pr)$_2$ (11mg, 16µL, 0.09 mmol, 3 equiv.) was added at 25 °C. Isothiocyanate **3** (15 mg, 0.03 mmol, 1 equiv.) was added and the mixture was stirred for 12 h. The mixture was diluted with CH_2Cl_2 (15 mL), washed with water (5 mL) and brine (2 x 5 mL), dehydrated (Na_2SO_4),

concentrated under reduced pressure, and purified by FCC (CH_2Cl_2/MeOH 98:2) to provide thiourea **SITC-Ahx** as a dark blue resin (16 mg, 86% yield).

TLC: R_f = 0.38 (CH_2Cl_2:MeOH = 95:5)

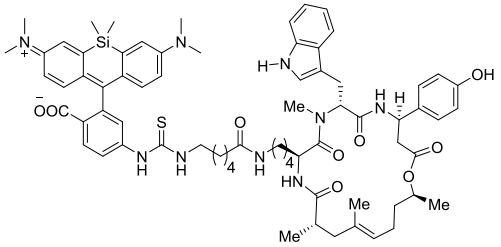
IR (ATR) $\tilde{\nu}$ = 2830 (w), 2397 (w), 2226 (w), 2159 (w), 2125 (w), 2022 (w), 1979 (w), 1901 (w), 1856 (w), 1816 (w), 1782 (w), 1664 (w), 1567 (m), 1465 (m), 1378 (w), 1302 (m), 1205 (m), 1149 (s), 889 (s), 800 (s), 756 (s), 679 (s), 629 (s) cm^{-1}.

^{1}H NMR ^{1}H NMR (400 MHz, MeOH-d_4, 297 K) δ = 7.86 (d, J = 8.5 Hz, 1H), 7.62 (d, J = 9.3 Hz, 2H), 7.05 (d, J = 2.8 Hz, 2H), 6.84 (d, J = 8.9 Hz, 2H), 6.72 – 6.54 (m, 2H), 3.63 (s, 5H), 2.97 (d, J = 1.8 Hz, 12H), 2.29 (t, J = 7.4 Hz, 2H), 1.73 – 1.53 (m, 4H), 1.36 (d, J = 7.6 Hz, 3H), 0.66 (s, 3H), 0.56 (s, 3H) ppm.

^{13}C{^{1}H} NMR (101 MHz, MeOH-d_4, 297 K) δ = 175.8, 172.7, 151.1, 146.9, 138.0, 132.9, 129.2, 126.9, 117.9, 114.8, 93.7, 52.0, 45.3, 40.5, 34.6, 29.4, 27.5, 25.6, 0.4, -1.4 ppm.

HRMS (ESI−TOF) m/z $[M]^+$ calculated for $C_{34}H_{43}N_4O_4SSi^+$ 631.2769; found: 631.2779.

SITC-Actin:



Boc-Lys-Ahx-Jasp-OH (10 mg, 0.011 mmol, 1 equiv.) was dissolved in CH_2Cl_2 (3 mL) and cooled to 0 °C. HCl in 1,4-dioxane (3 mL, 4 M) was added and reaction mixture was stirred at 0 °C for 2 h. Volatiles were evaporated by first applying a flow of nitrogen, then high vacuum. The residue obtained was dissolved in DMF (3 mL), cooled to 0 °C and EtN(i-Pr)$_2$ (8 mg, 11 µL, 0.066 mmol, 6 equiv.) was added. Isothiocyanate **3** (11 mg, 0.022 mmol, 2 equiv.) dissolved in CH_2Cl_2 (500 µL) was added and the mixture was stirred for 16 h, letting it slowly reach 25 °C. The mixture was diluted with CH_2Cl_2 (15 mL), washed with saturated solution of $NaHCO_3$ (5 mL) and brine (5 mL), dehydrated (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by preparative HPLC (C18) to give **SITC-Actin** as a blueish-green colored resinous solid (8.9 mg, 64% yield).

Method used for preparative HPLC: isocratic: 2 min (30% MeCN in H_2O) + 1%AcOH, → gradient over 38 min (30-100% MeCN in H_2O) + 1% AcOH, → 10 min 100% MeCN + 1% AcOH. Flow 25 mL/min.

TLC: R_f = (CH_2Cl_2:MeOH = 9:1).

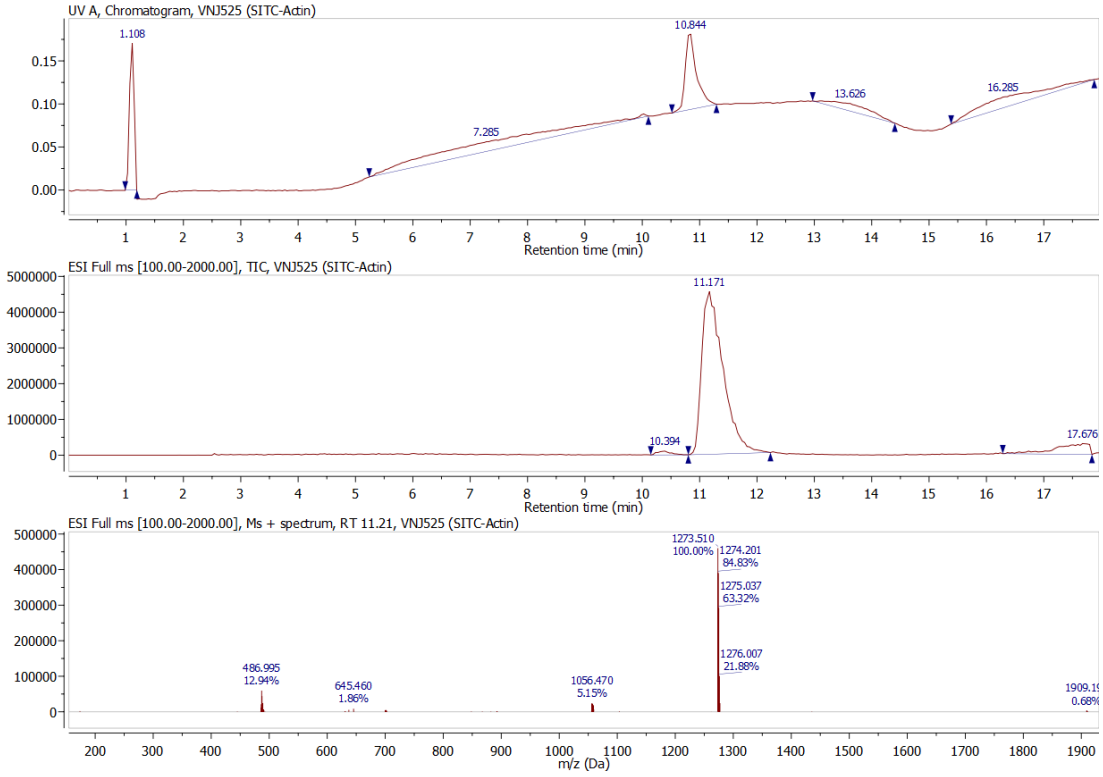
$[\alpha]_{20}$D = +9.7 (c = 1, $CHCl_3$:MeOH = 1:1).

IR (ATR) $\tilde{\nu}$ = 2881, (s), 2356, (w), 2223, (w), 2156, (w), 2062, (w), 2001, (w), 1962, (w), 1693, (m), 1620, (s), 1571, (m), 1402, (s), 1303, (s), 1192, (s), 1148, (s), 1075, (s), 861, (s), 800, (s), 722, (s), 646, (s) cm^{-1}.

^{1}H NMR (600 MHz, DMSO-d_6, 297 K) δ = 10.82 (s, 1H), 9.31 (s, 1H), 8.87 – 8.28 (m, 2H), 7.79 (d, J = 1.8 Hz, 1H), 7.76 – 7.57 (m, 6H), 7.56 – 7.50 (m, 1H), 7.51 – 7.41 (m, 1H), 7.39 – 7.30 (m, 2H), 7.30 – 7.16 (m, 3H), 7.16 – 6.88 (m, 9H), 6.73 (s, 7H), 5.58 – 5.27 (m, 2H), 5.30 – 5.01 (m, 1H), 4.92 (s, 1H), 4.48 (dd, J = 75.0, 2.2 Hz, 3H), 3.40 (s, 1H), 3.01 (t, J = 6.0 Hz, 3H), 2.91 (s, 15H), 2.02 (dt, J = 7.6, 3.5 Hz, 4H), 1.88 (s, 4H), 1.67 – 1.52 (m, 4H), 1.47 (s, 7H), 1.32 (s, 2H), 1.23 (s, 12H), 1.15 (d, J = 6.4 Hz, 2H), 1.14 – 0.99 (m, 6H), 0.97 – 0.89 (m, 5H), 0.85 (d, J = 6.3 Hz, 5H), 0.70 (d, J = 6.9 Hz, 1H), 0.63 (s, 4H), 0.56 (d, J = 6.2 Hz, 2H), 0.51 (s, 3H), 0.49 (d, J = 6.5 Hz, 1H) ppm.

^{13}C{^{1}H} NMR (150 MHz, DMSO-d_6, 297 K) δ = 174.7, 170.1, 156.7, 149.6, 136.6, 136.6, 136.0, 131.6, 128.9, 128.7, 128.6, 128.5, 128.5, 128.2, 128.1, 128.0, 128.0, 127.7, 127.5, 127.5, 126.1, 126.0, 125.6, 125.4, 123.9, 123.8, 122.0, 121.3, 118.5, 116.8, 116.8, 115.5, 114.1, 109.9, 98.9, 90.7, 88.1, 81.5, 78.3, 71.4, 64.7, 59.7, 44.1, 35.8, 35.3, 30.9, 29.5, 29.1, 28.7, 28.4, 26.6, 25.5, 23.5, 22.6, 22.3, 21.7, 20.6, 20.1, 19.9, 18.4, 17.3, 17.2, 16.1, 15.0, 14.4, 0.4, -0.7 ppm.

HRMS (ESI−TOF) m/z $[M]^+$ calculated for $C_{71}H_{90}N_9O_9SSi^+$ 1272.6346; found: 1272.6345.



4. Photophysical characterization

UV-Vis absorption spectra were recorded on a Jasco V780 double beam spectrophotometer in the range of 250-800 nm. Fluorescence emission spectra were recorded on a Edinburgh Intruments FLS980 emission spectrometer using a Peltier-cooled PMT detector. Emission quantum yields were determined in an absolute fashion on the same spectrometer using an integrating sphere sample unit. Si-rhodamines were excited at 600 nm. pH values were recorded using a Mettler-Toledo FiveEasy™ standard pH meter with a Mettler-Toledo InLab® Micro-Pro-ISM pH electrode. Data analysis and visualisation was achieved using the programming language Python (Python Software Foundation,

https://www.python.org) (including the NumPy[15] and Matplotlib[16] libraries) and the free scientific plotting package Veusz (https://veusz.github.io).

Novel Si-rhodamines were investigated in aqueous phosphate citrate buffer[17] (pH 3.0) with a resulting pH of 3.14, complemented with 20% DMSO. SiR-COOH was studied in TBS buffer employing 50 mM Tris and 150 mM NaCl with 20% DMSO, adjusted to pH 7.4.

The pK_a values were approximated from UV-Vis absorption and fluorescent emission titrations. To a mixture of 6.75 mL of 0.1 M HCl, 0.75 mL DMSO and 10-30 µL of dye stock (depending on the stock concentration and the dye) was added aqueous NaOH (with 10% DMSO added) in small quantities. After every addition of base, the pH value of the solution as well as the emission and absorption spectra were recorded. The absorption maxima were plotted against the corresponding pH value and the slopes fitted by means of a generalized logistic function or an exponential function to approximate the inflection point and hence, the pK_a value.

5. Microscopy data acquisition and processing

All fluorescence microscopy images were acquired with a commercial Zeiss Elyra S.1 system (Zeiss, Germany), using a Zeiss plan-apochromat 63×x/1.40 Oil DIC objective (Zeiss, Germany) and an Andor iXon 885 emCCD (Andor-Oxford Instruments, UK). The Zeiss "Zen" software (version 2.1, Zeiss, Germany) was used for image acquisition and reconstruction of SIM images. The Elyra's 642 nm (100 mW) and 405 nm (50 mW) laser lines have been used for excitation of SITC/SiR dyes and DAPI, respectively.

Exposure time and laser intensity were adjusted to utilize the full dynamic range of the emCCD when possible. An exposure time of 200 ms and an em-gain of 50 were selected for enhanced sensitivity of the camera. Laser power was set to 20% (fixed cells, SiR-Actin), 50% (fixed cells, SITC-Actin), and 24% (DAPI), respectively. In case of live cells, a laser intensity of 75% was used with SITC-Actin and 25% with SiR-Actin, with a 200 ms exposure time in both cases.

For imaging in the microscope, cultured HeLa cells (routinely cultured in DMEM, supplemented with 10% FBS and 1% Pen/Strep at 37°C and 5% CO_2) were transferred to 8-well plates (ibidi µ-Slide with Glass Bottom #1.5H, ibidi GmbH, Germany) the day prior to fixation or live cell imaging.

Fixation was performed for 10 minutes in 3.5% PFA, heated to 37°C, followed by washing in PBS.

6. Cell culture and cytotoxicity testing

MCF10A (ATCC CRL-10317) were plated in 96-well plates (5000 cells/well) in DMEM/F-12 medium (DMEM + 5% horse serum, 1% Pen/Strep, 10µg/ml human insulin, 0.1 µg/ml Cholera enterotoxin, 20 ng/ml human EGF, 05 µg/ml hydrocortison) and incubated at 5%CO_2, 37°C. On the next day, cells were treated with compounds as indicated, transferred to an automated incubator-housed imaging system (Incucyte S3, Sartorius) and cultivated for 6 days. Every three hours a phase contrast image was generated and the confluency calculated.

7. Actin *in vitro* experiments

For the *in vitro* actin polymerization assay fluorescence-based pyrene labelled muscle actin assay from Cytoskeleton, Inc was used, and supplier's protocols were followed with modifications specific to our needs (Actin Polymerization Biochem Kit™, Cat. # BK003) https://www.cytoskeleton.com/. Dye to protein (G-actin) ratio in used kit is 0.6.

Detailed protocol information:

Test compounds were dissolved in non-polymerizing buffer provided by the supplier as "G-Buffer" (5 mM Tris-HCl pH 8.0, 0.2 mM $CaCl_2$, 0.2 mM ATP) by pipetting DMSO primary stock solutions of

test compounds giving secondary stock solutions. Black, 384-well plates were used for measurement (Well plate reader: Tecan, The Infinite 200 PRO).

G-Buffer preparation:

1. An aliquot of ATP (# BSA04, Cytoskeleton, Inc.) was kept on ice until it liquified. (Previously stored at -80 °C). After thawing, it was centrifuged for 5 s in a microcentrifuge to collect the liquid at the bottom of the tube.

2. G buffer was prepared by adding 2.0 µL ATP (# BSA04, Cytoskeleton, Inc.) per 1.0 mL of "General Actin Buffer" (# BSA01, Cytoskeleton, Inc.).

Actin polymerization buffer:

500 mM KCl, 20 mM $MgCl_2$, 0.05 M guanidine carbonate and 10 mM ATP reconstituted with 2 ml of 100 mM Tris-HCl pH 7.5. Aliquot into 200 µl volumes, snap freeze in liquid nitrogen and store at -70°C.

Pyrene-G-actin solution preparation:

1. 1 mL of cold water was added to 1 mg of pyrene labelled G-actin (# AP05 Cytoskeleton, Inc.) and suspension was divided to aliquots of 5 µL. Aliquots were frozen in liquid nitrogen and stored at -80 °C.

2. G-actin stock solution was prepared by dissolving an aliquot of pyrene actin (5 µL) in 356 µL of cooled G buffer (concentration of actin is 0.25 mg/mL). Solution was mixed by pipetting up and down and placed on ice for one hour (in order to depolymerize actin oligomers). If several aliquots of actin were used, they were transferred to a common vial after diluting.

3. G-actin stock solution was centrifuged at 4 °C and 14000 rpm/min for 30 minutes.

4. The supernatant was transferred to a new vial and kept on ice. (**pyrene-G-actin solution)**

Pyrene-F-actin solution preparation:

1. G-actin stock solution was prepared by dissolving an aliquot of pyrene actin (5 µL) in 356 µL of cooled G buffer (concentration of actin is 0.25 mg/mL).

2. 10 uL of actin polymerization buffer was added and solution in tube was left at room temperature for one hour to polymerize.

Sample preparation:

1. 1.0 mM stock solutions of the SiR-Actin, SITC-Actin, SiR-COOH, jasplakinolide+SiR-COOH (each 1 mM) or only jasplakinolide, were prepared in DMSO as well (positive control) – **MainStock**(s).

2. DMSO primary stock is made by pipetting of DMSO to G buffer in ratio 2:15. This can be also used to dilute the concentrations of the PrimaryStocks for concentration series. - **DMSO-PS**.

3. MainStocks of the SiR-Actin, SITC-Actin and jasplakinolide+SiR-COOH were diluted with G buffer to obtain 118 µM solution (primary stock). (20 µL of the MainStock was dissolved in 150 µL G buffer) – **Test-PS (jasplakinolide-PS, jasplakinolide+SiR-COOH-PS, SiR-Actin-PS or SITC-Actin-PS)**.

Carrying out the assay:

All experiments were carried out as triplicates determinations. A blank value and a reference value for jasplakinolide (positive control and for referencing) were recorded.

a) Induction of polymerization (actin polymerization assay)

G-actin was diluted according manufacturer's protocol to a concentration of 5 µM in G-buffer and secondary stocks of compounds were pipetted to give final concentration of 20 µM, keeping the final concentration of DMSO at 2%, for testing of induction of polymerization (Figure S9, b-c). After shaking for 10 seconds using excitation at 360 nm and detection at 410 nm, fluorescence intensity was measured every 60 seconds over 4 h, at 37 °C. Experiments were done in triplicates and mean values are shown. Slopes were calculated in the linear range of plots and compared in relative to jasplakinolide.

16 wells were used for the actin polymerization assay:

Well 1-3: pyrene-G-actin + SiR-COOH-PS ("baseline reference")

Well 4-6: pyrene-G-actin + **jasplakinolide-SiR-COOH-PS** ("positive control reference")

Well 7-9: pyrene-G-actin + **SiR-Actin-PS** ("test")

Well 10-12: pyrene-G-actin + **SITC-Actin-PS** ("test")

50 µL pyrene G actin solution was pipetted into each well (1-12). To wells 4-12 each was added 10 µL of **Test-PS** solutions or **SiR-COOH-PS** (1-3).

The 384 well plate prepared in this way was placed in the plate reader and the measurement was initiated.

Well plate reader measurement parameters:

Measurement type:	Kinetic	120 cycles, 60 s interval time
Fluorescence reading from:	Top-Mode	
Fluorescence wavelengths:	Excitation	350 nm ± 20 nm
	Emission	410 nm ± 20 nm
Temperature:	37 °C	
Gain:	50	
Number of Flashes:	3	

Integration Time:	40 µs
Lag Time:	0 µs
Settle Time:	0 ms
Mirror:	50% Mirror
Shaking:	5 s (orbital) Amplitude: 1 mm

To evaluate the data, the values of the triple determinations were averaged. The intensity of emitted enhanced fluorescence of the reference wells was subtracted from the intensity of emitted fluorescence of tested compounds. The slope k was determined from the data obtained in the range of the first 50 minutes. For this purpose, a linear regression according to $y = kx + c$ ($c = 0$) was carried out (Using Origin(Pro), Version 2019b, OriginLab Corporation, Northampton, MA, USA).

b) Actin depolymerization assay

Pyrene-F-actin solution (50 µL) was pippeted into black 96-well plate followed by 10 uL of **Test -PS** solutions, jasplakinolide-PS, SiR-Actin-PS, SITC-Actin-PS or DMSO-PS (5 µM of protein and 20 µM of ligands) and incubated for 1 h at room temperature to allow diffusion into the fibers. Actin depolymerization was initiated by dilution with G-buffer (150 µL) and decay of pyrene fluorescence was followed over time of 5 h (After shaking for 10 seconds using excitation at 360 nm and detection at 410 nm, fluorescence intensity was measured every 60 seconds over 4 h, at 37 °C). All measurements were performed as triplicates and mean values were plotted without normalization (Figure S9, a).

c) Kinetics of fluorogenicity:

Test-solutions for assaying kinetics of fluorogenicity were prepared by 4x dilution of Test-PS solutions with G-buffer.

Pyrene-F-actin solution aliquots (50 µL) were added to wells of a black 96-well plate followed by 10 uL of **Test-solutions**, SiR-Actin-PS, SITC-Actin-PS or DMSO-PS (5 µM protein concentration and 1.25 µM ligand concentration). Change in fluorescence intensity of SiR-Actin and SITC-Actin was followed over time (2.5 h). After shaking for 10 seconds using excitation at 620 nm and detection at 670 nm, fluorescence intensity was measured every 60 seconds over 2.5 h, at 37 °C.

8. References

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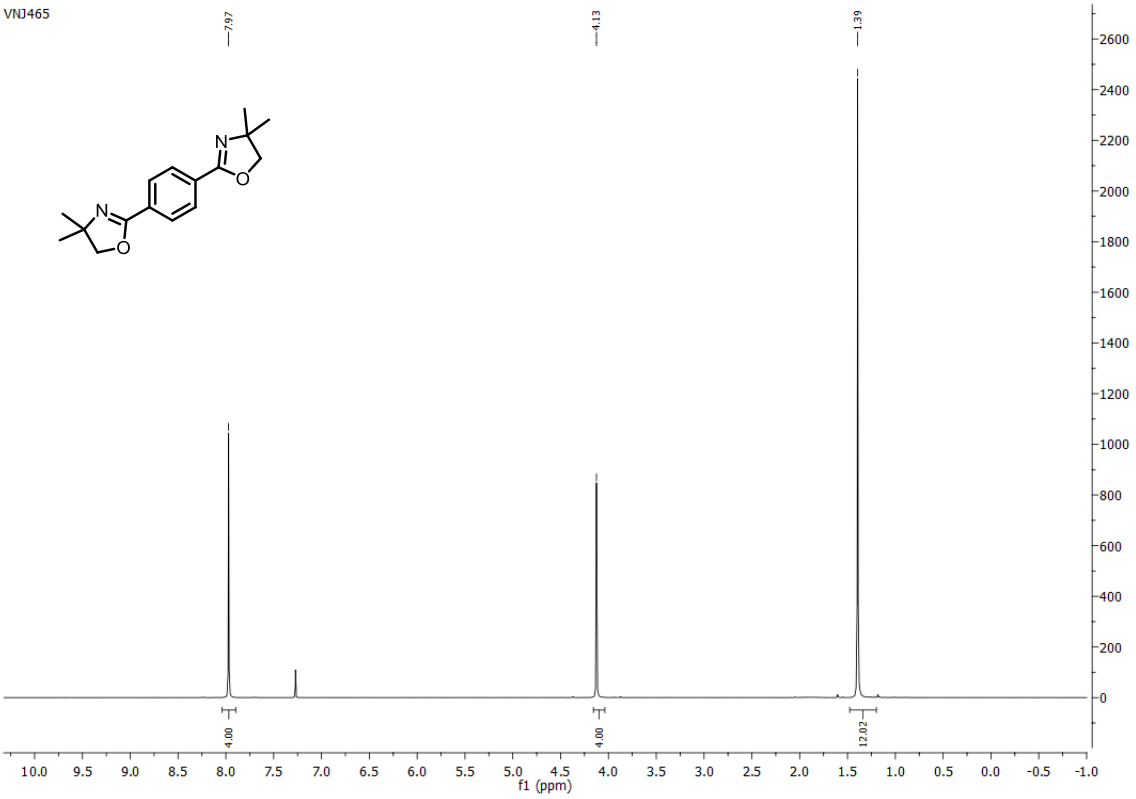
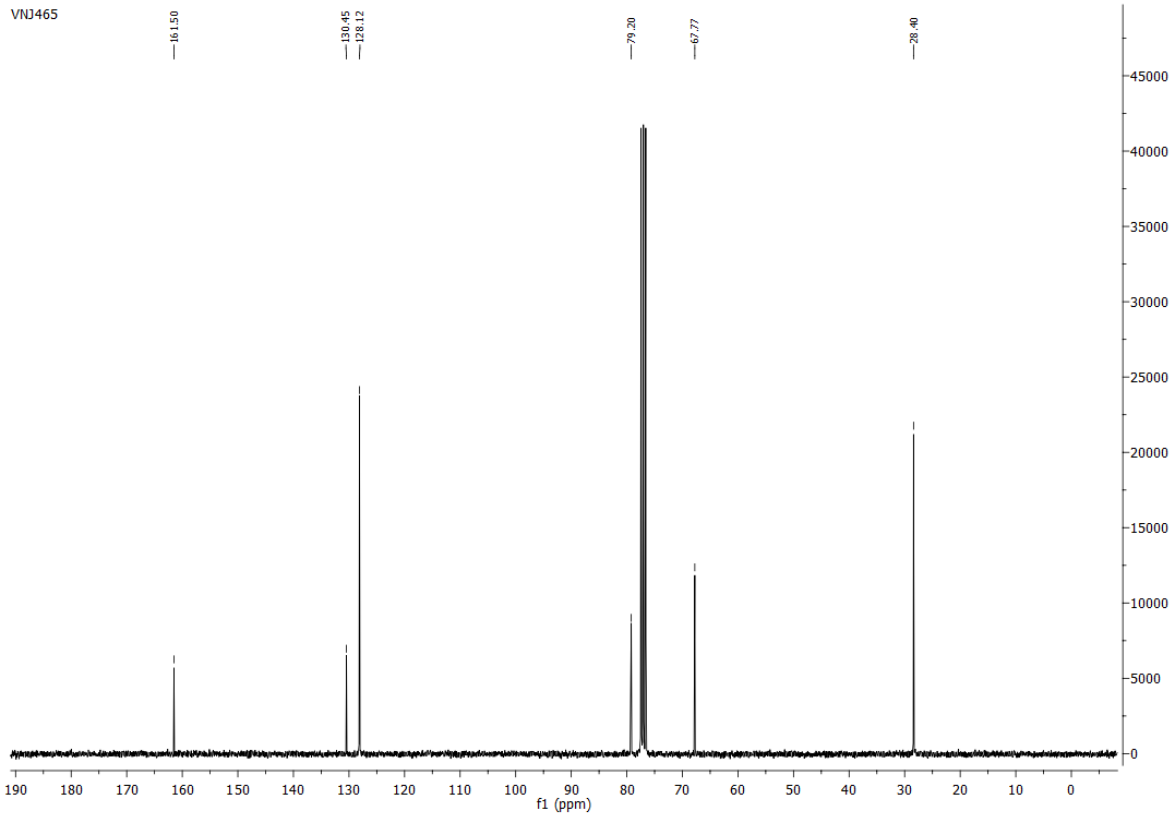
9. NMR spectra

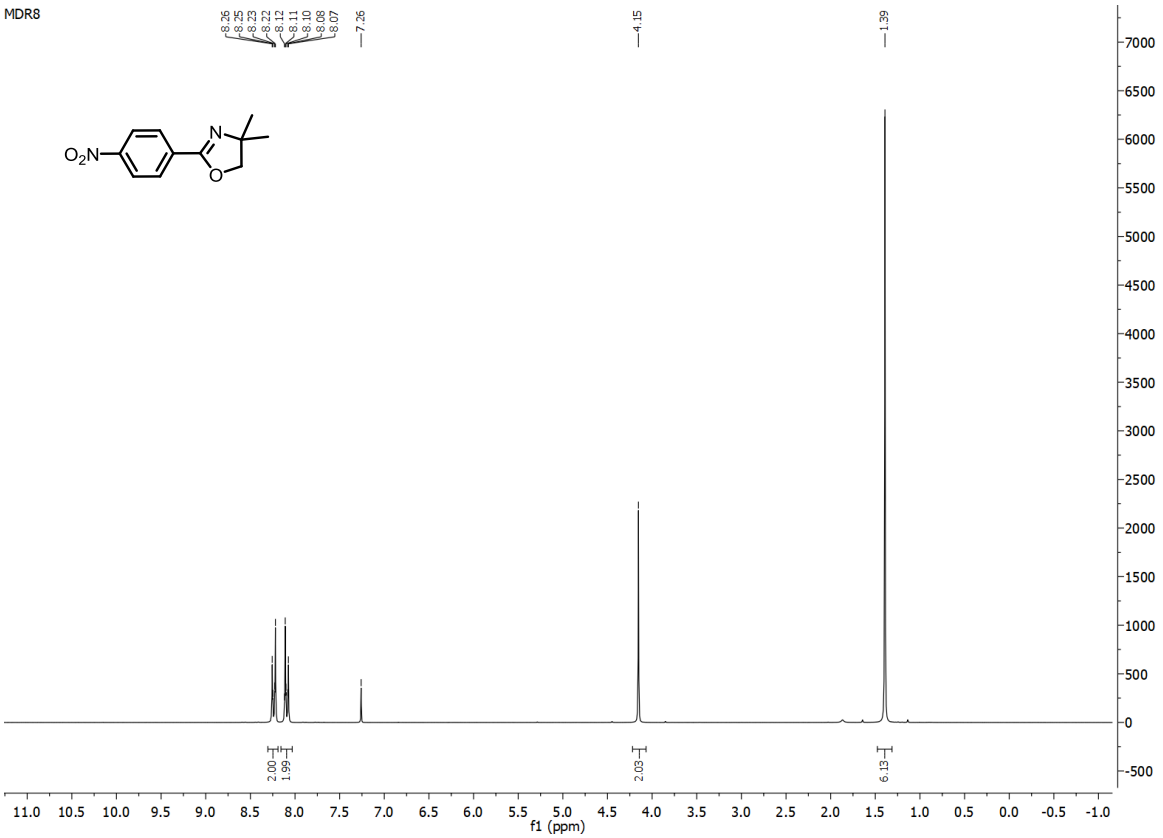
^{1}H NMR spectrum of 6a: 300 MHz, $CDCl_3$, 297 K



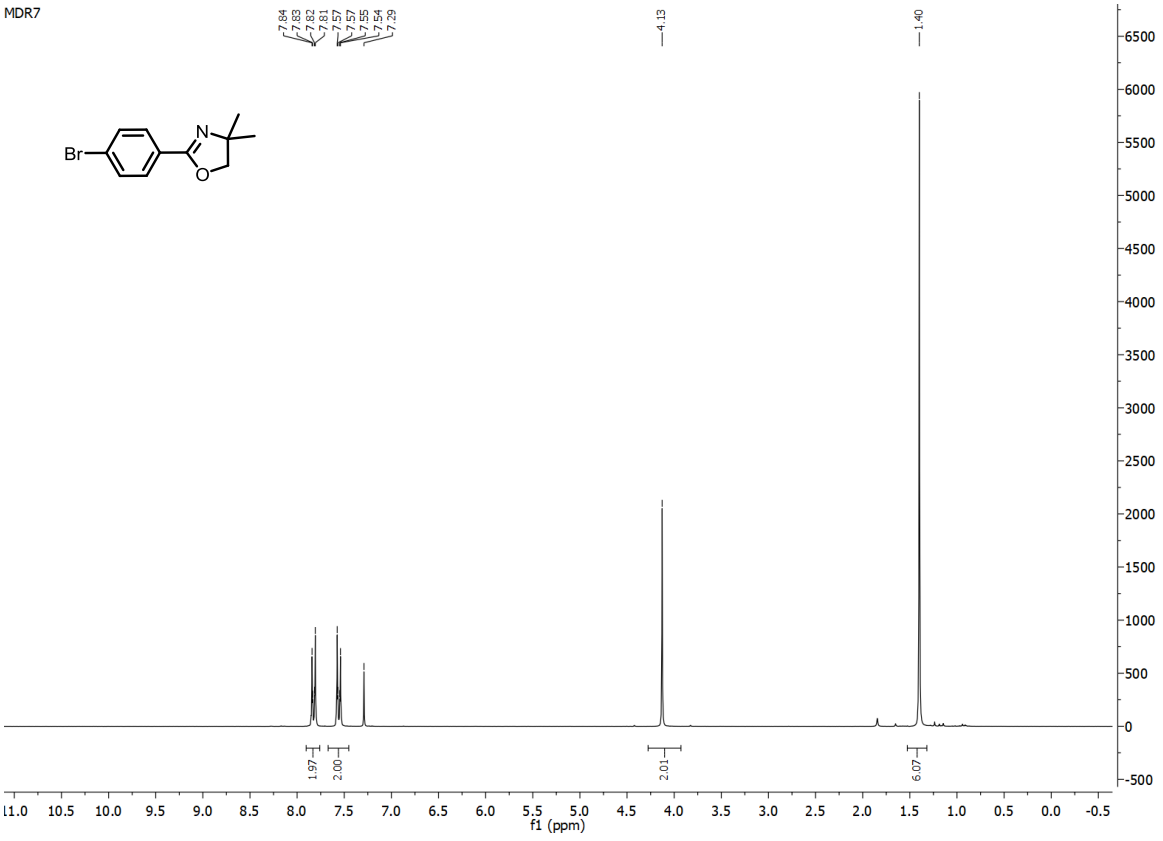
^{13}C{^{1}H} NMR spectrum of 6a: 75 MHz, $CDCl_3$, 297 K



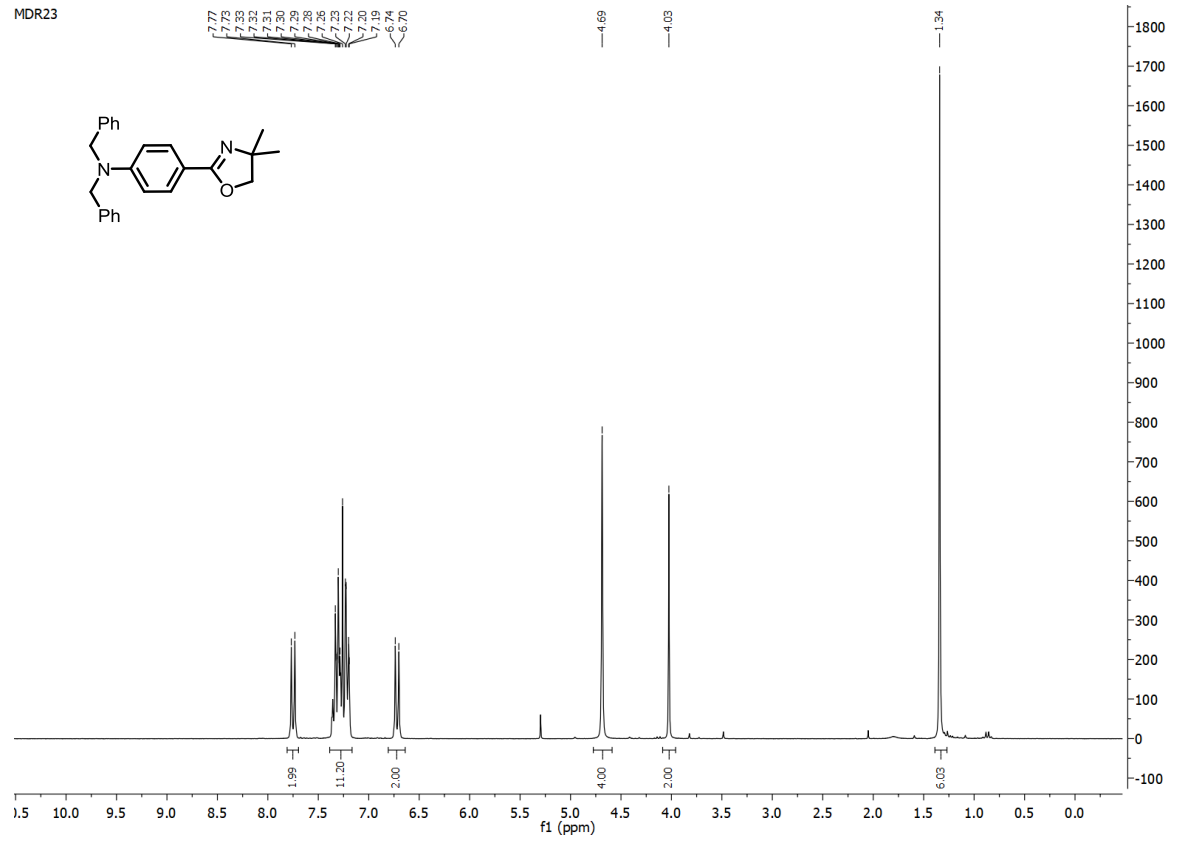
^{1}H NMR spectrum of compound 6b: 250 MHz, Chloroform-*d*, 297 K



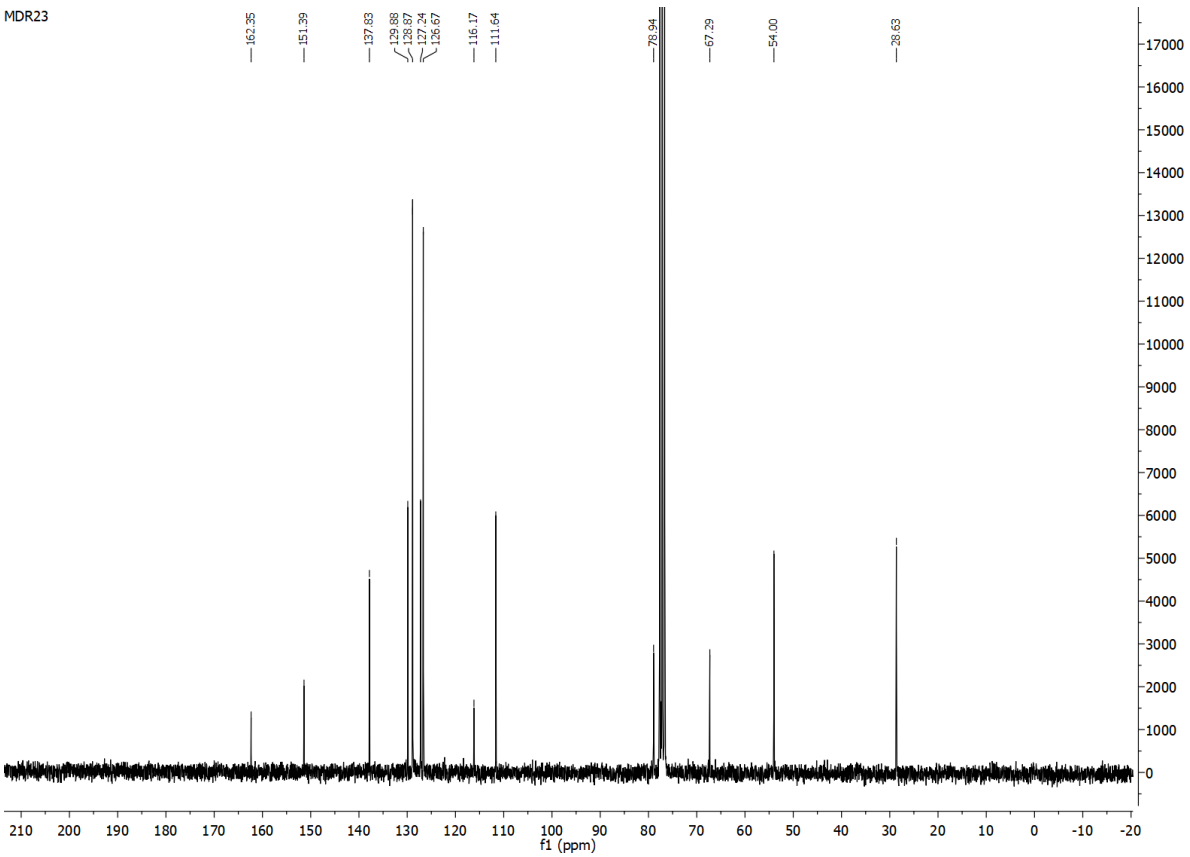
^{1}H NMR spectrum of compound 6c: 250 MHz, Chloroform-*d*, 297 K



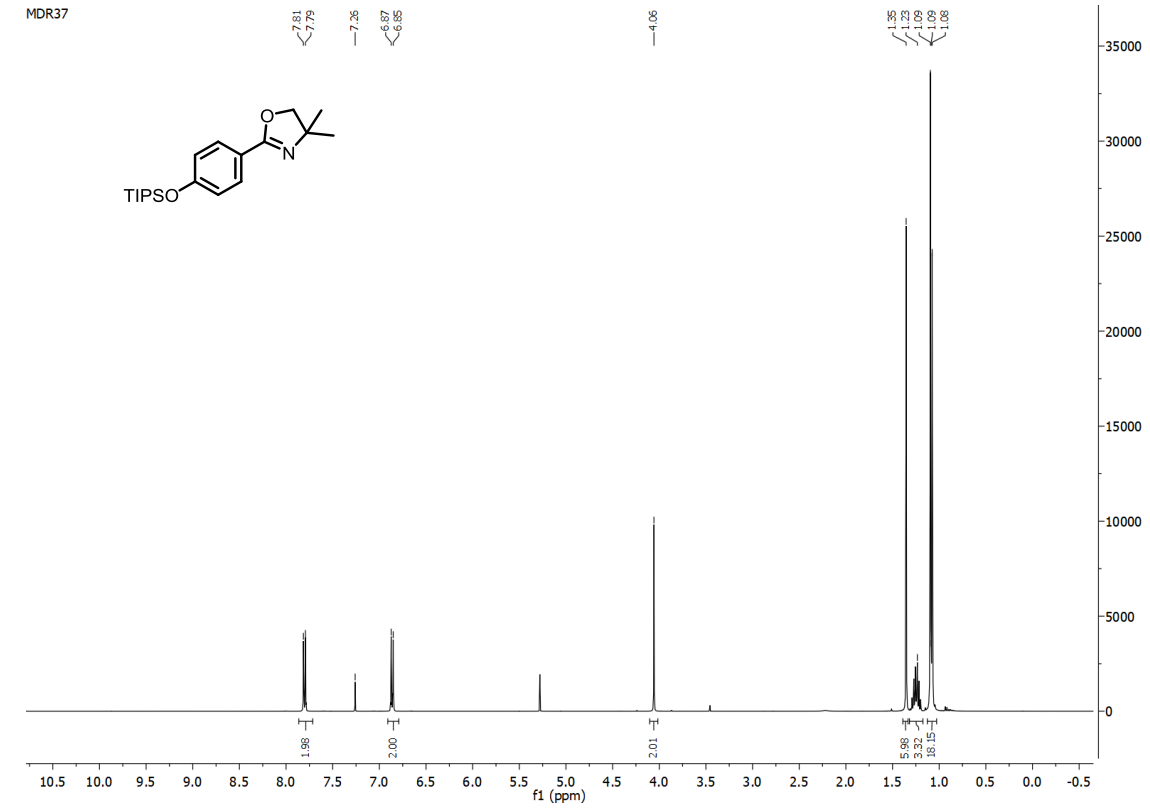
^{1}H NMR spectrum of compound 6d: 250 MHz, Chloroform-*d*, 297 K



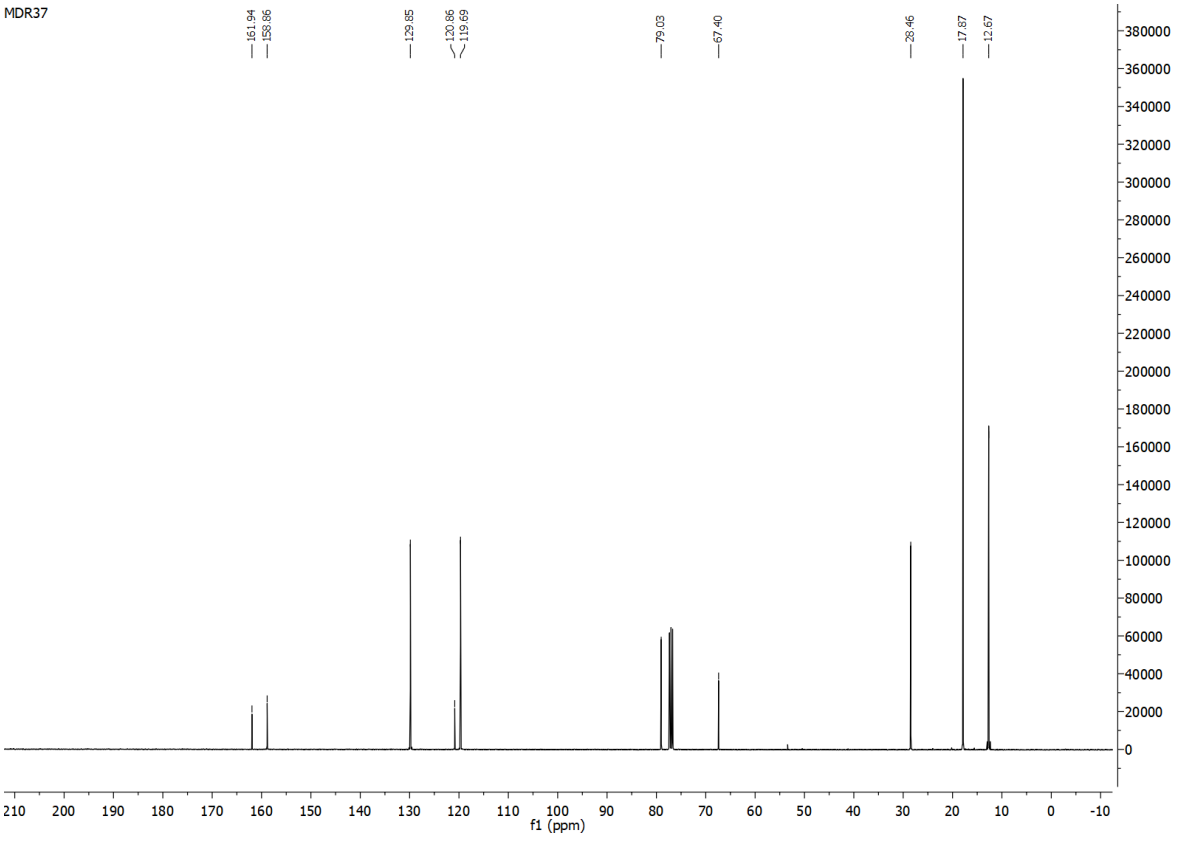
^{13}C{^{1}H} spectrum of compound 6d: 63 MHz, Chloroform-*d*, 297 K



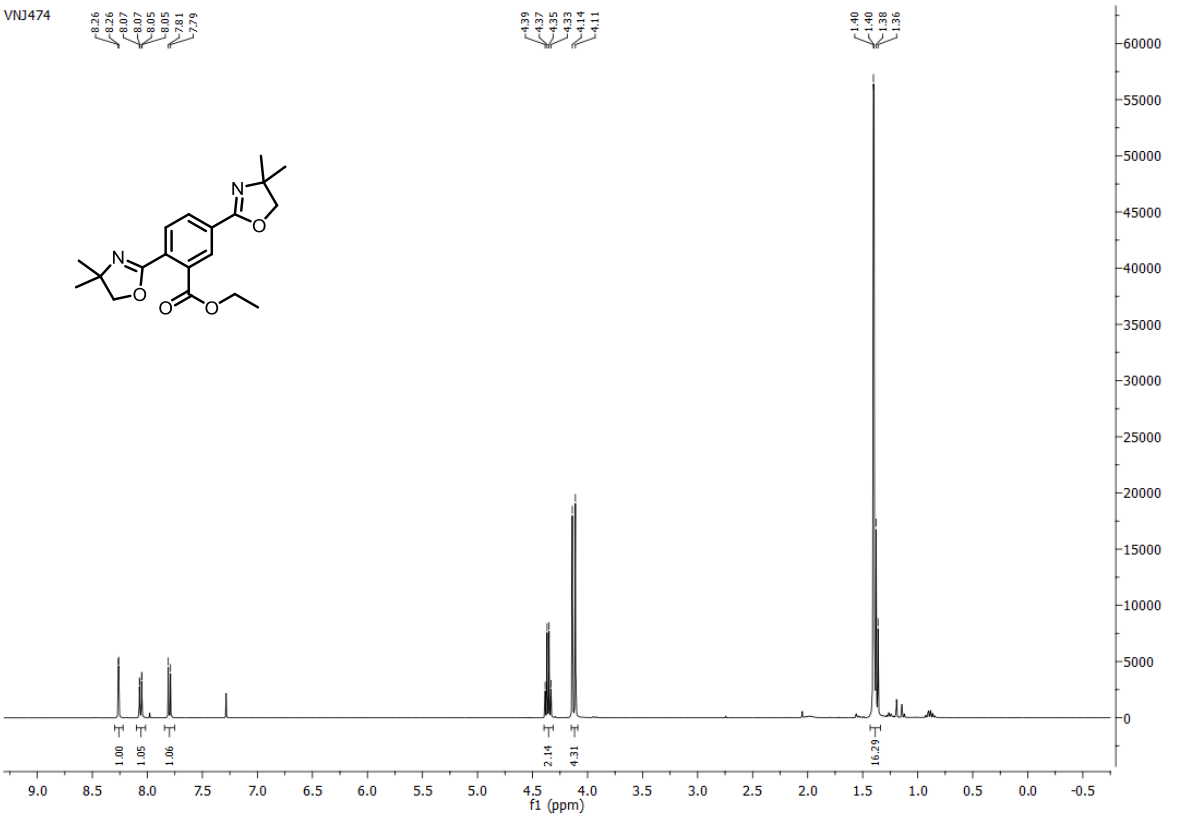
^{1}H NMR spectrum of compound 6e: 400 MHz, Chloroform-*d*, 297



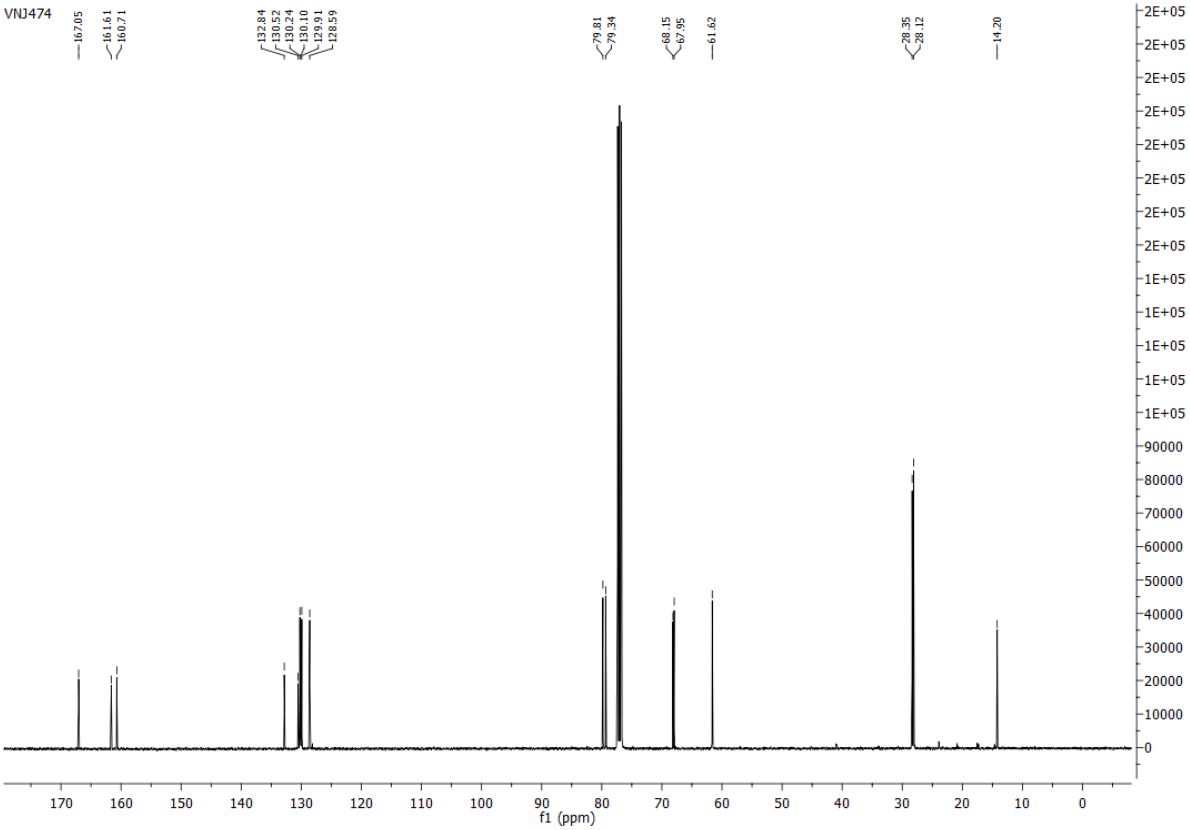
^{13}C{^{1}H} spectrum of compound 6e: 101 MHz, Chloroform-*d*, 297



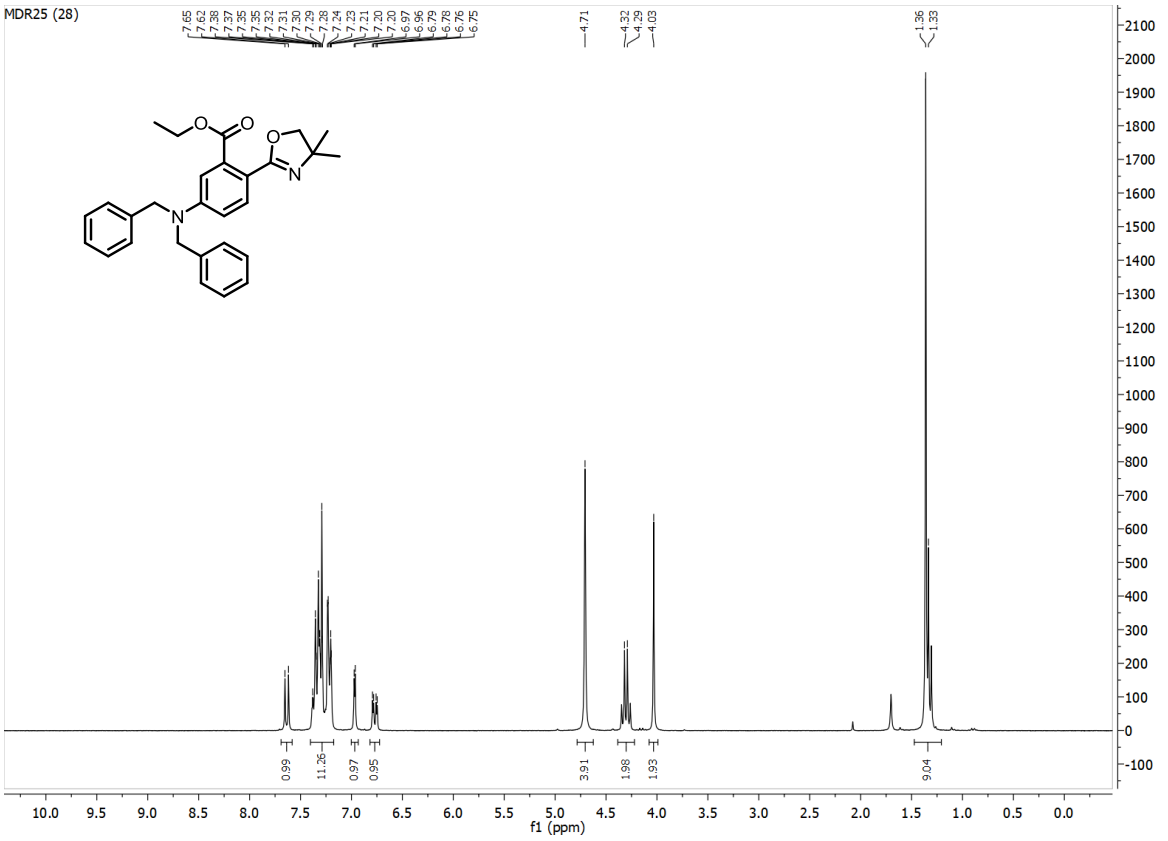
^{1}H NMR spectrum of compound 5a: 400 MHz, Chloroform-*d*, 297 K



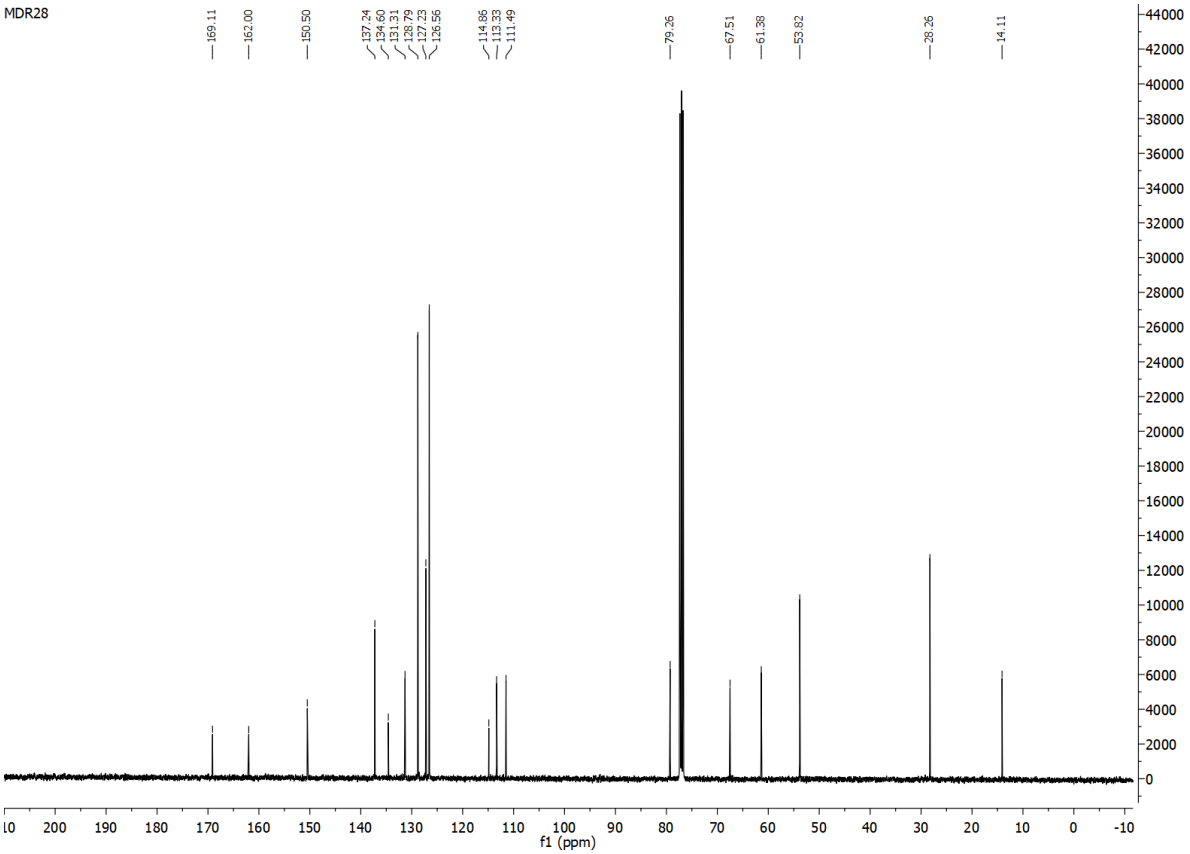
^{13}C{^{1}H} NMR spectrum of compound 5a: 101 MHz, Chloroform-*d*, 297 K



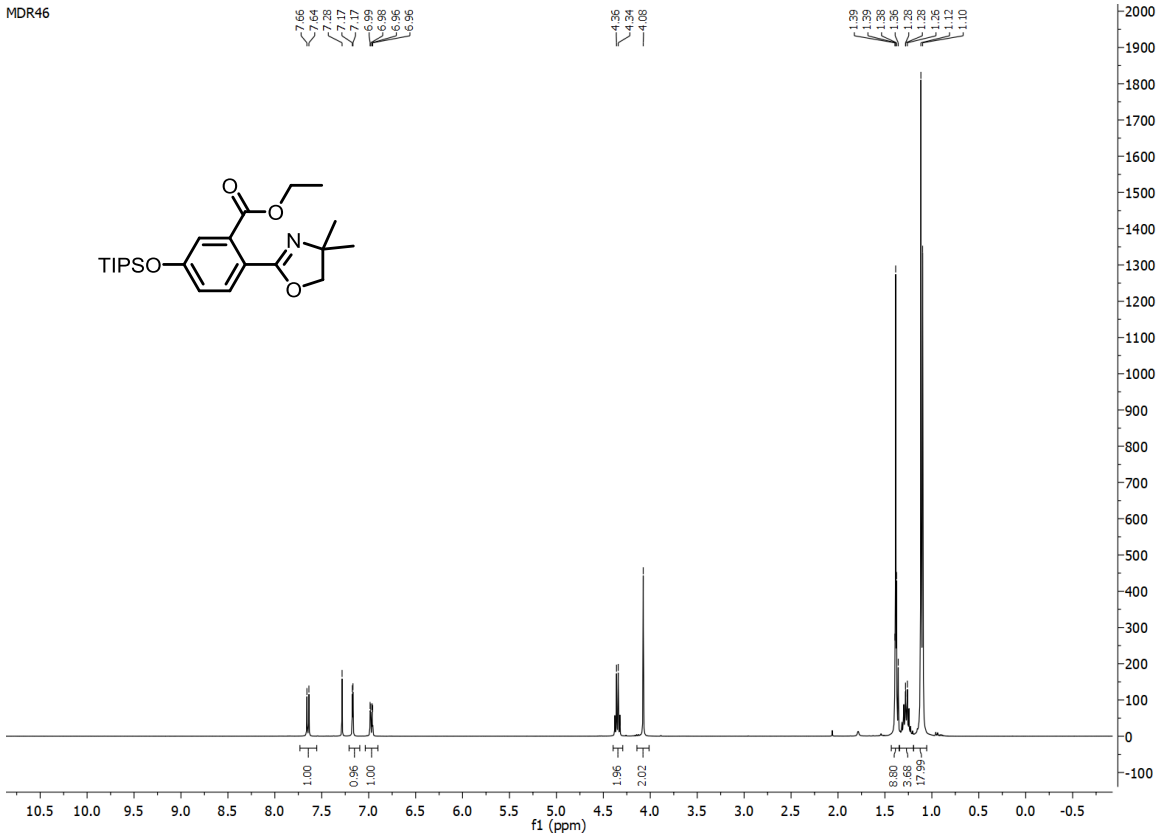
^{1}H NMR spectrum of compound 5b: 400 MHz, Chloroform-*d*, 297 K



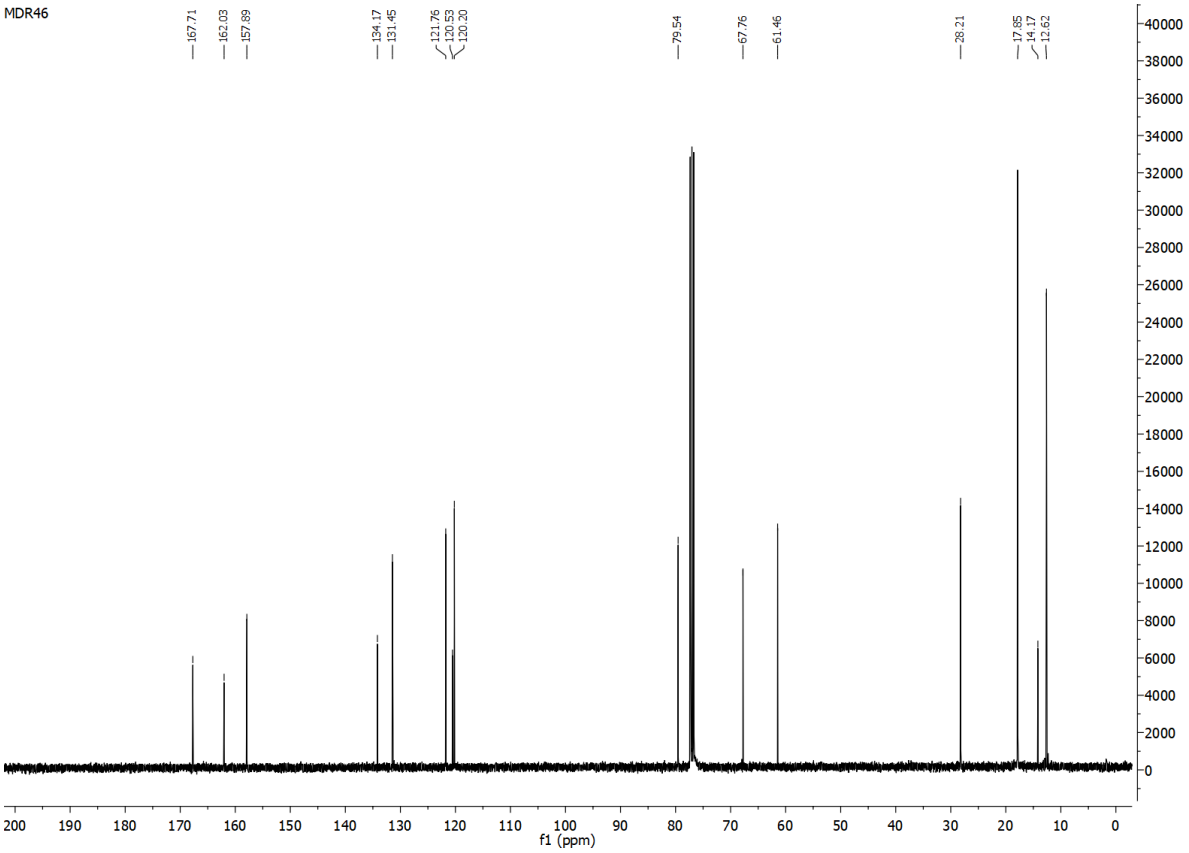
^{13}C{^{1}H} spectrum of compound 5b: 101 MHz, Chloroform-*d*, 297 K



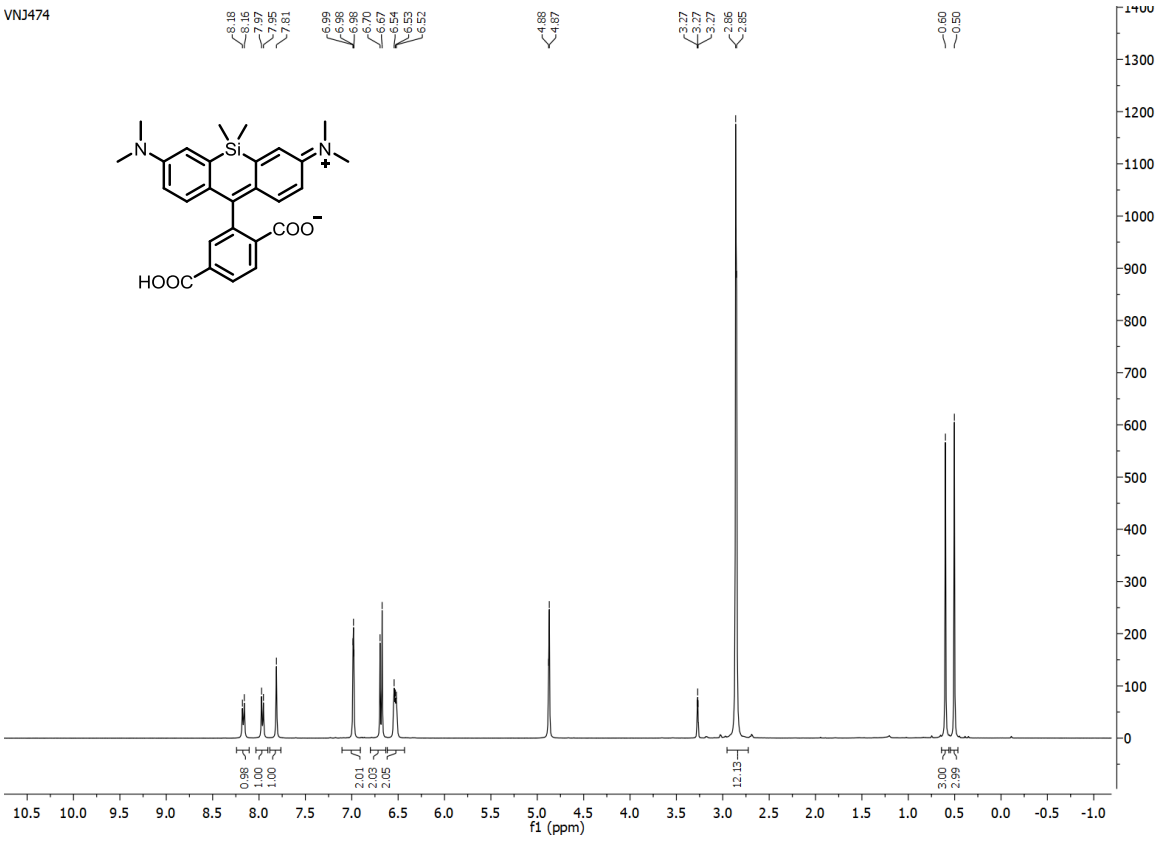
^{1}H NMR spectrum of compound 5c: 400 MHz, Chloroform-*d*, 297 K



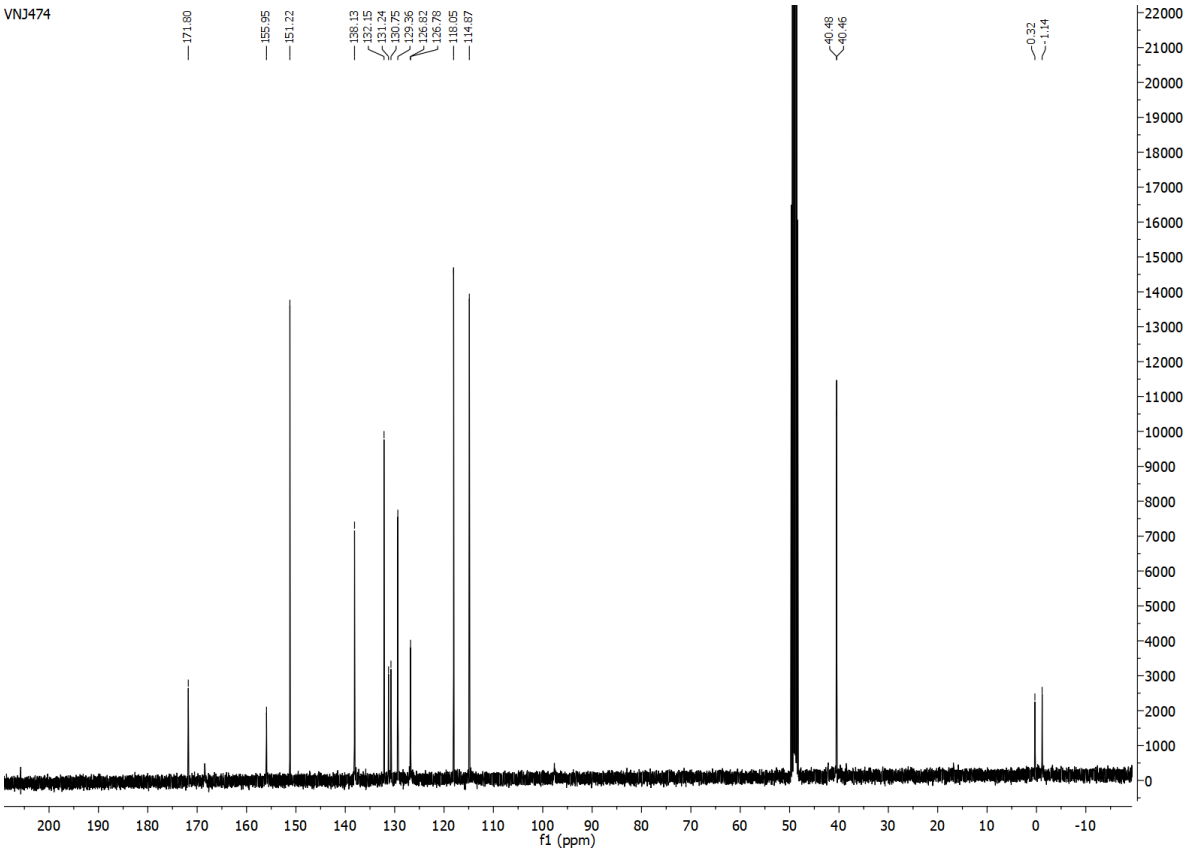
^{13}C{^{1}H} spectrum of compound 5c: 101 MHz, Chloroform-*d*, 297 K



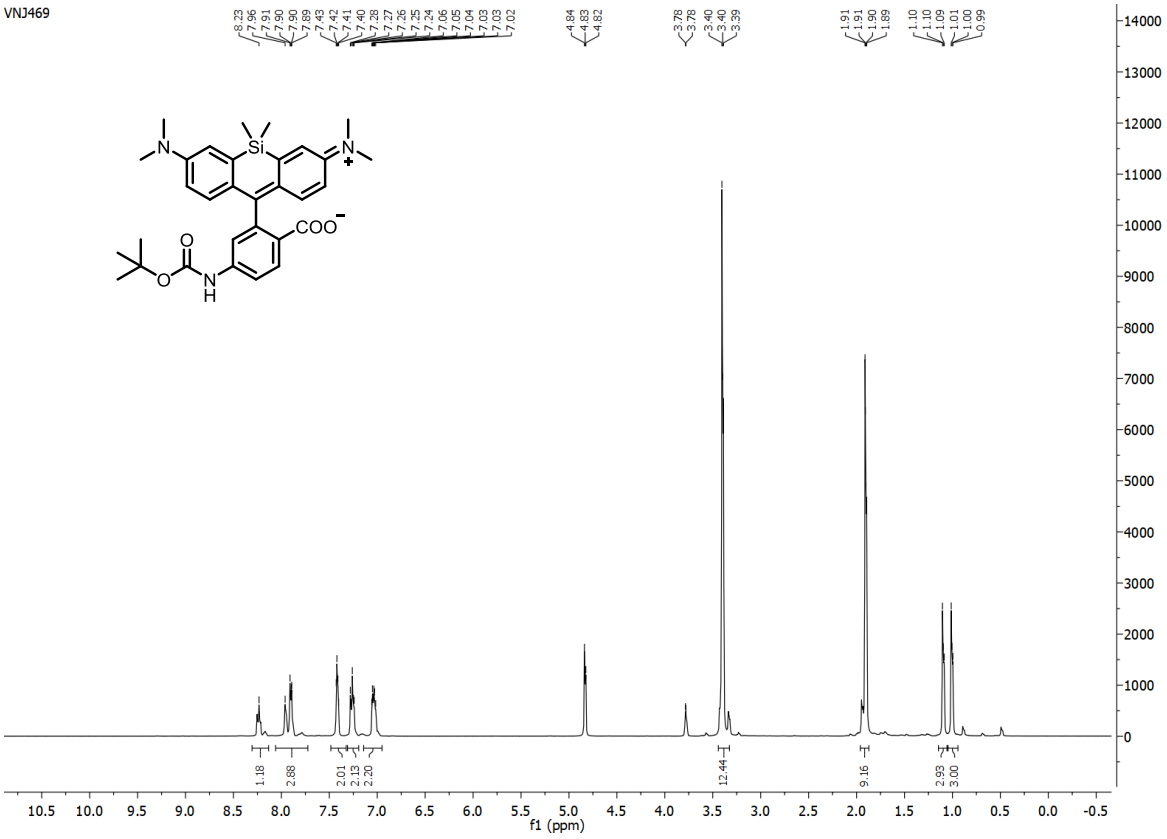
^{1}H NMR spectrum of compound 2: 400 MHz, Methanol-*d4*, 297 K



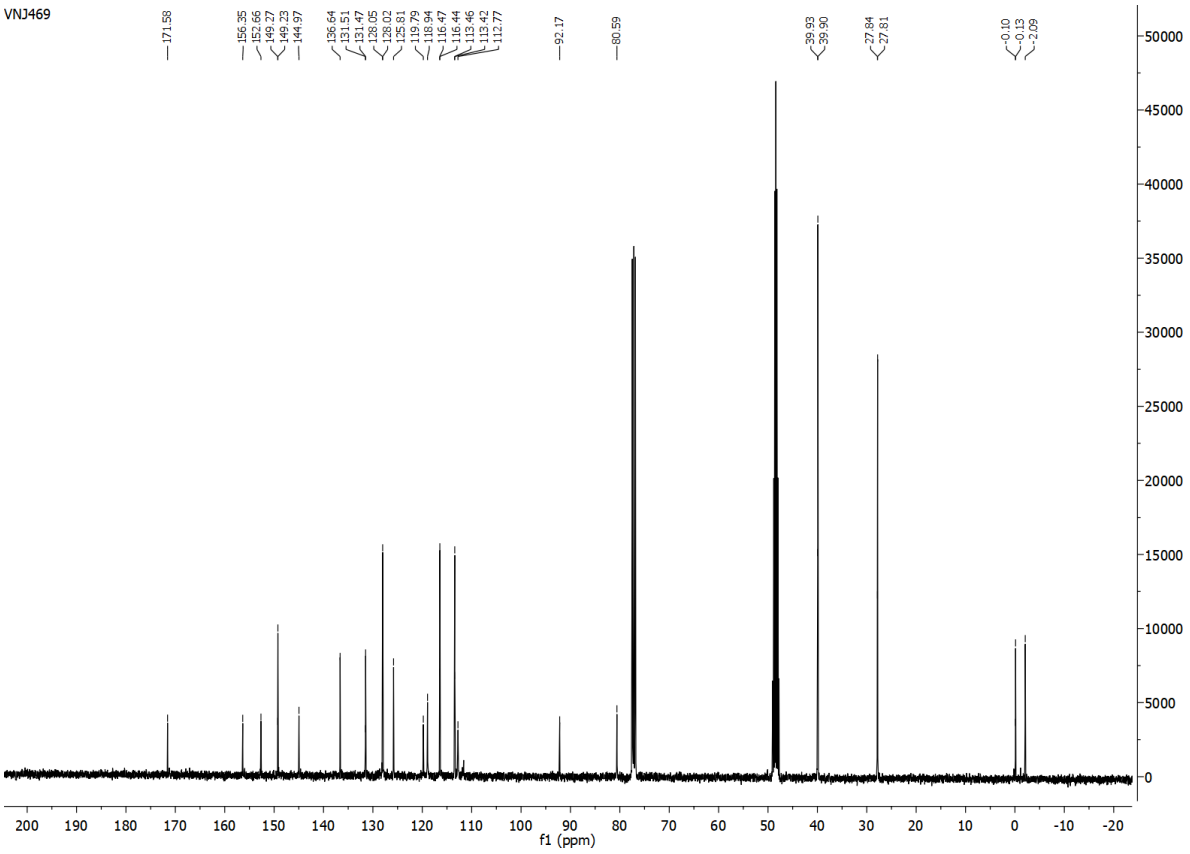
^{13}C{^{1}H} NMR spectrum of compound 2: 101 MHz, Methanol-*d4*, 297 K



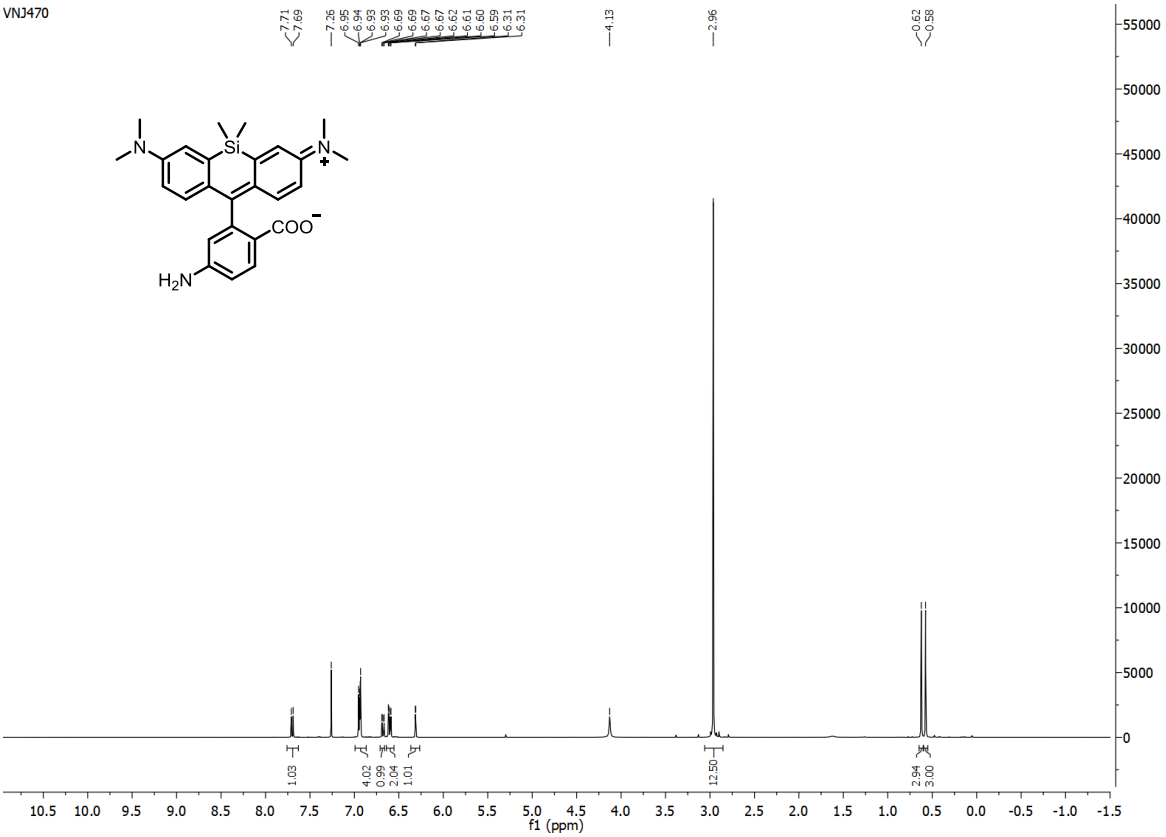
^{1}H NMR spectrum of compound 8: 400 MHz, $CDCl_3$:Methanol-*d4* = 1:1, 297 K



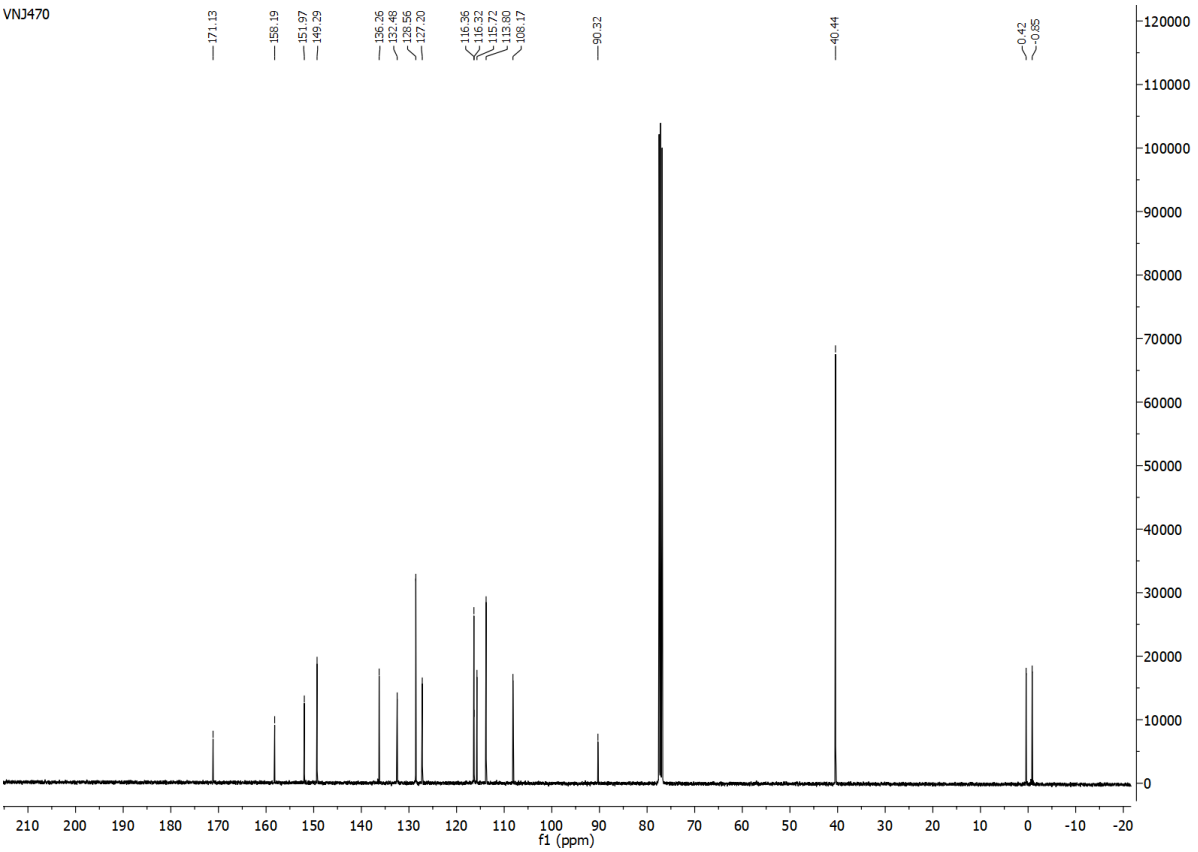
^{13}C{^{1}H} NMR spectrum of compound 8: 101 MHz, $CDCl_3$:Methanol-*d4* = 1:1, 297 K



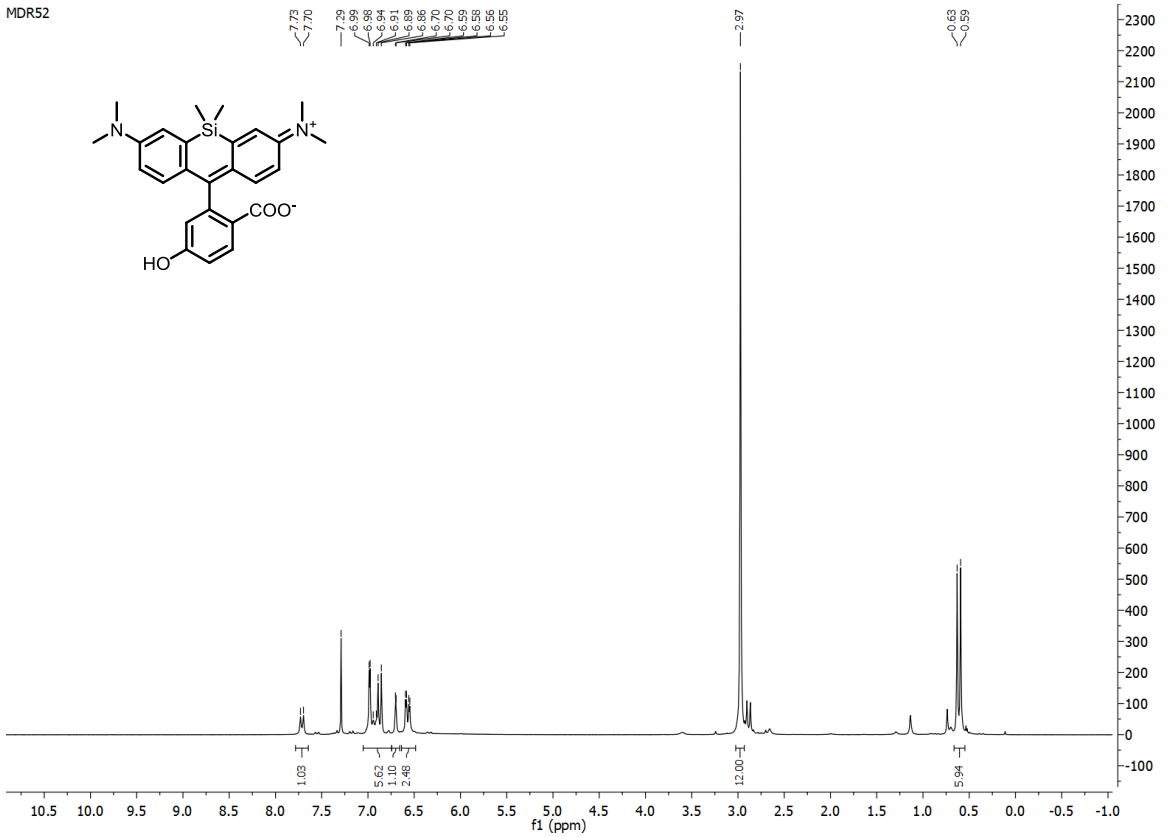
^{1}H NMR spectrum of compound 9: 400 MHz, Chloroform-*d*, 297 K



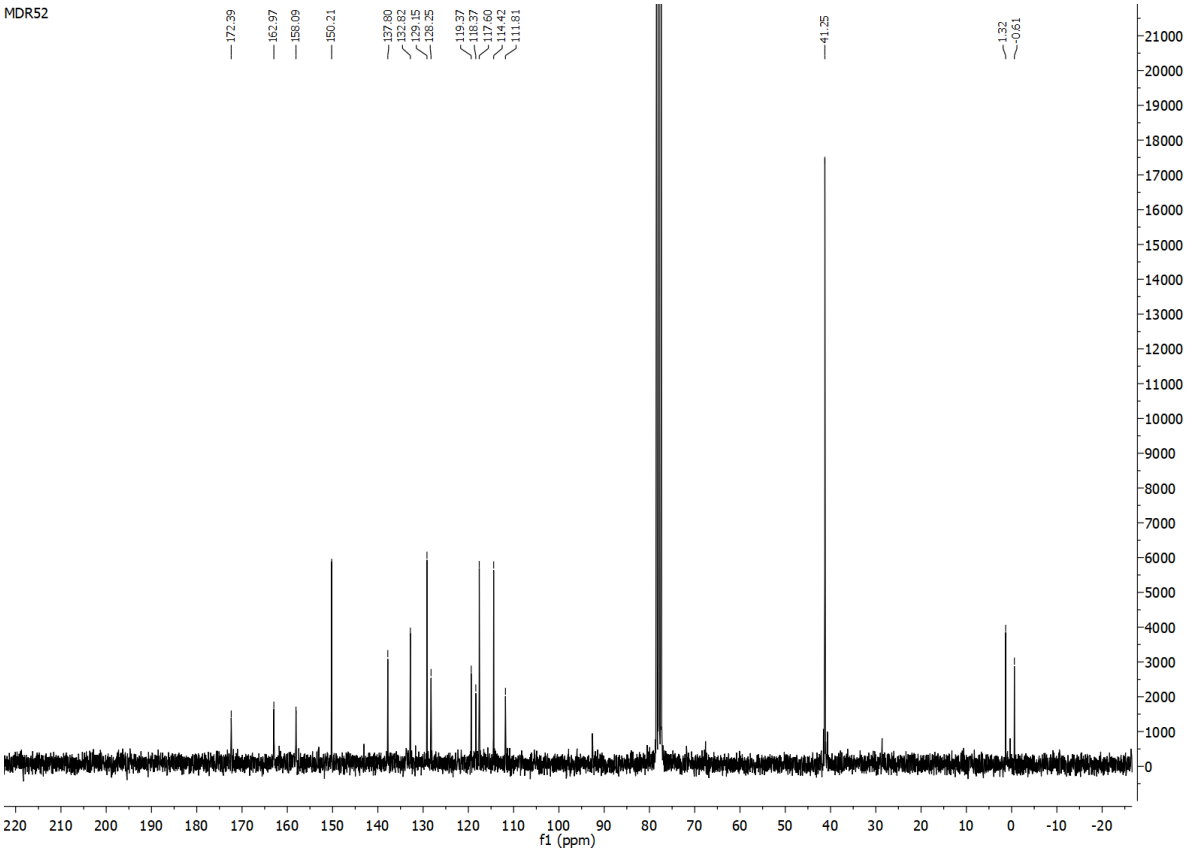
^{13}C{^{1}H} NMR spectrum of 9: 101 MHz, Chloroform-*d*, 297 K



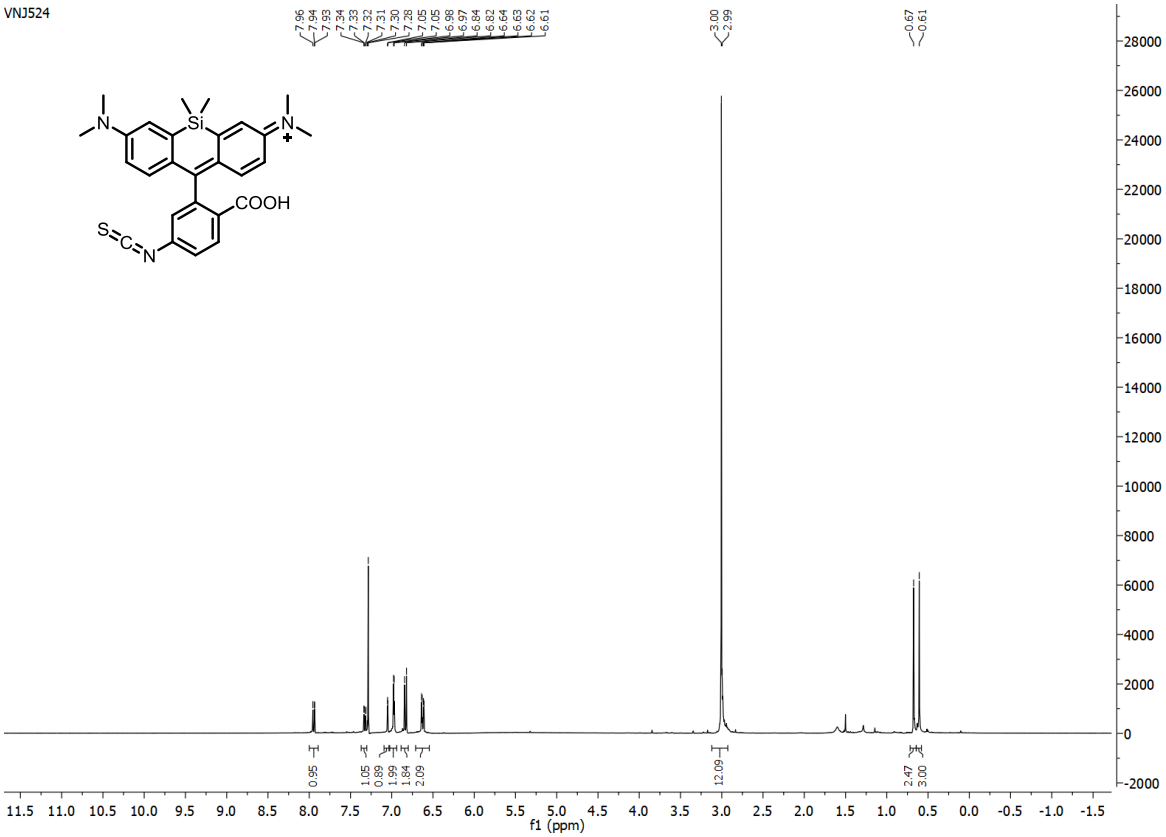
^{1}H NMR spectrum of compound 10: 250 MHz, Chloroform-*d*, 297 K



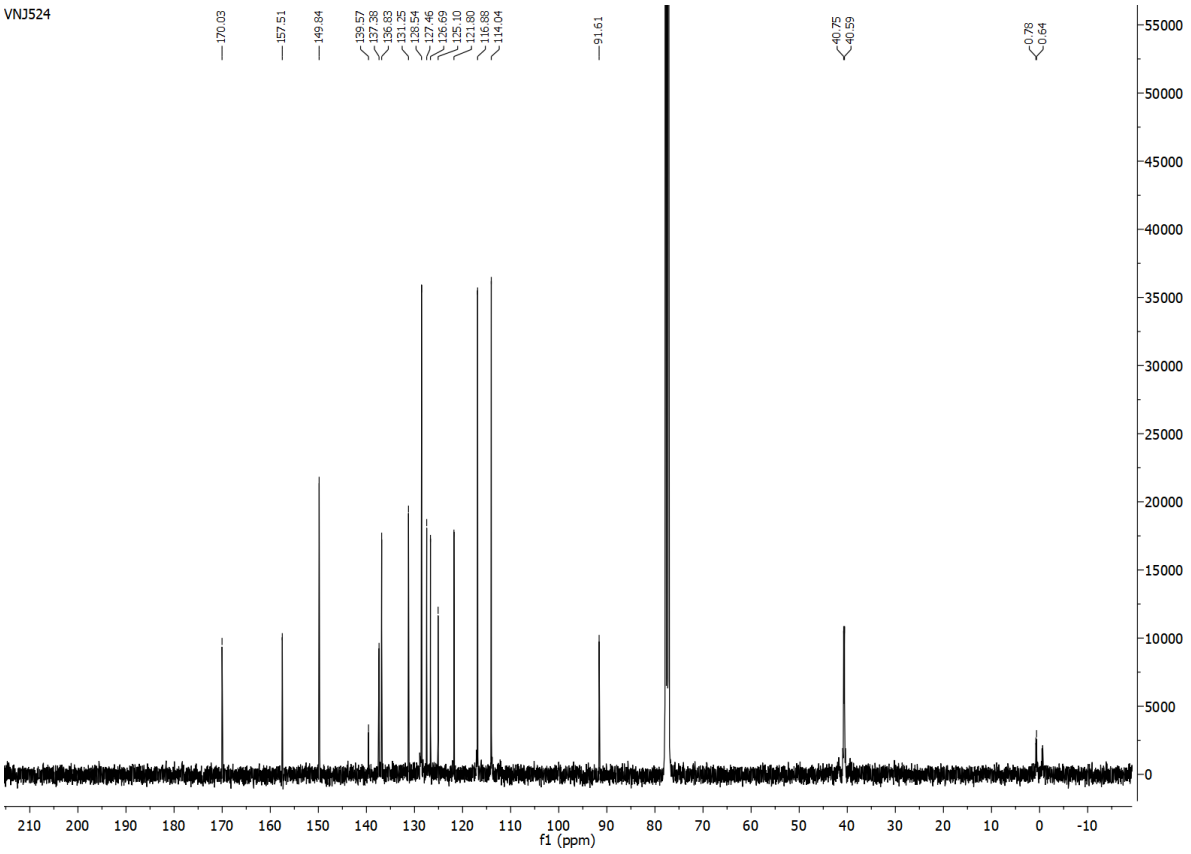
^{13}C{^{1}H} spectrum of compound 10: 63 MHz, Chloroform-*d*, 297 K



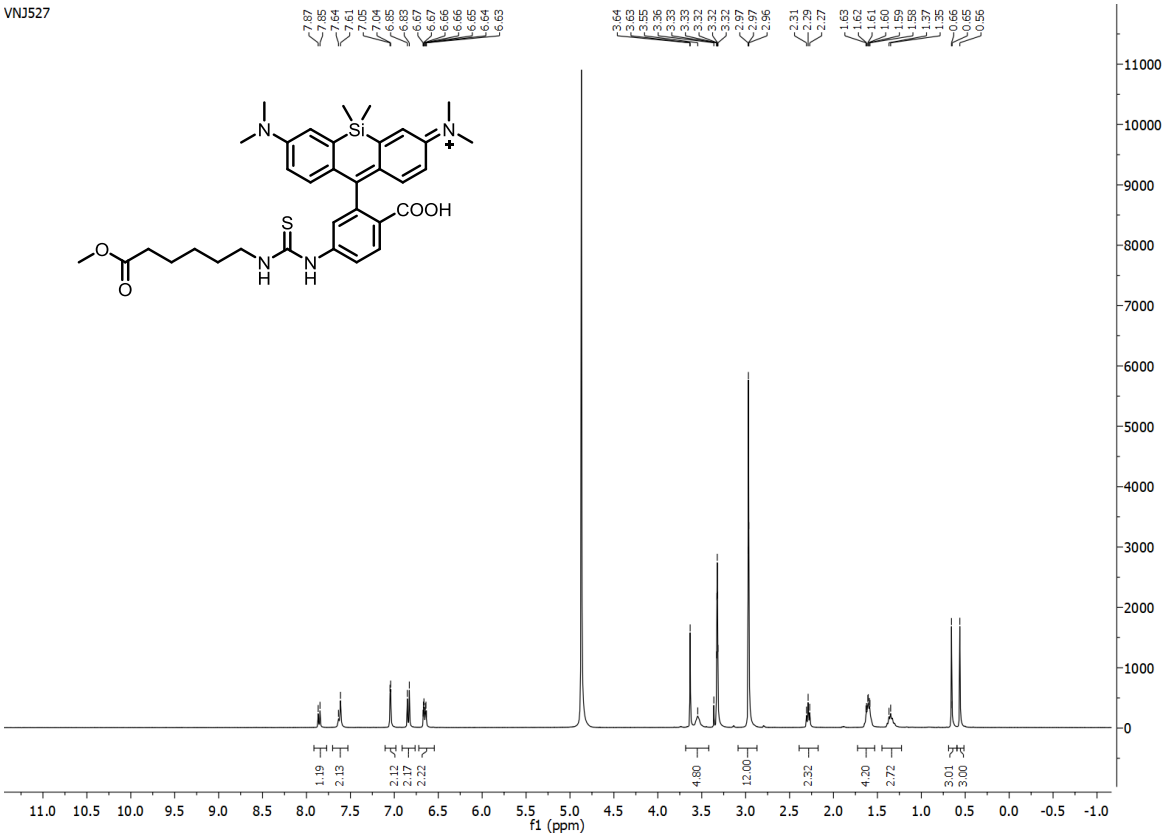
^{1}H NMR spectrum of compound 3: 400 MHz, Chloroform-*d*, 297 K



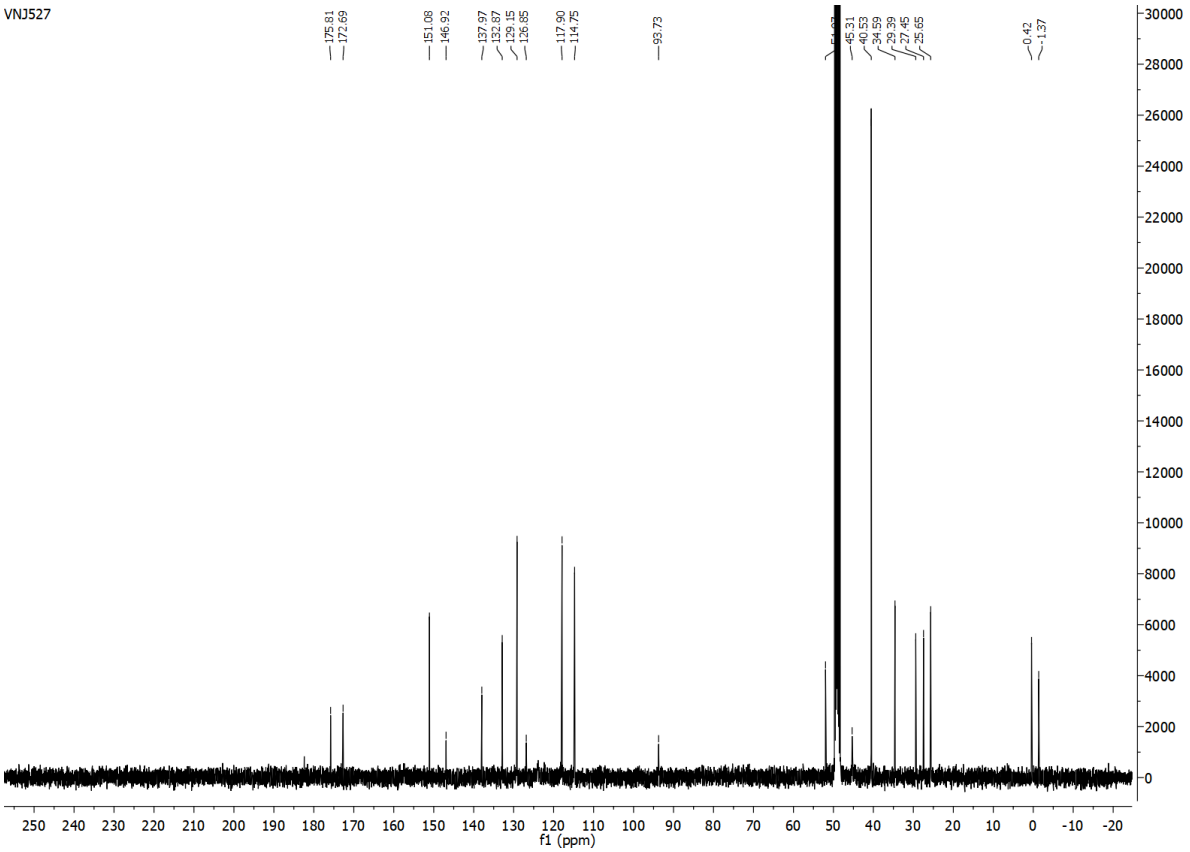
^{13}C{^{1}H} NMR spectrum of compound 3: 101 MHz, Chloroform-*d*, 297 K



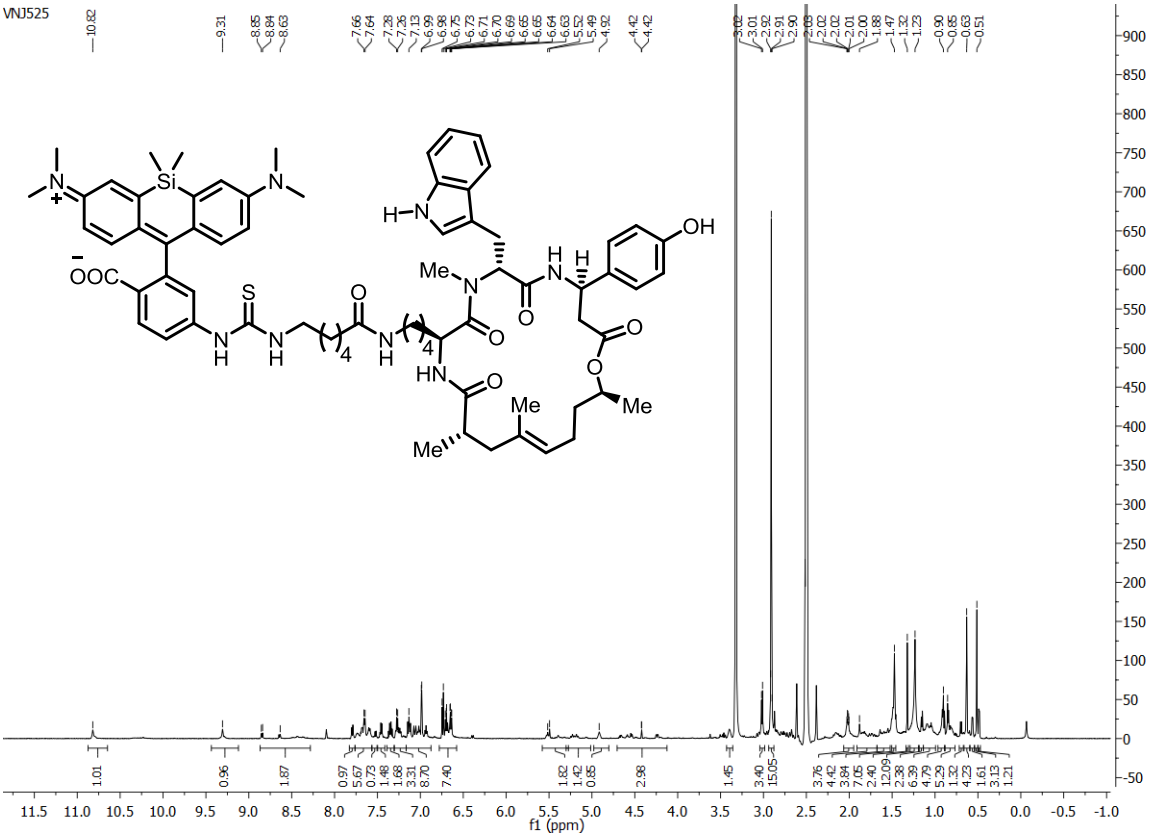
^{1}H NMR spectrum of SITC-Ahx: 400 MHz, Methanol-d_4, 297 K



^{13}C{^{1}H} NMR spectrum of SITC-Ahx: 101 MHz, Methanol-d_4, 297 K



^{1}H NMR spectrum of SITC-Actin: 600 MHz, DMSO-d_6, 297 K



^{13}C{^{1}H} NMR spectrum of SITC-Actin: 151 MHz, DMSO-d_6, 297 K